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Dimethylation of eEF1A at Lysine 55 Plays a Key Role in the Regulation of eEF1A2 on Malignant Cell Functions of Acute Myeloid Leukemia

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Abstract

Objective: This study aimed to explore whether eukaryotic translation elongation factor 1 alpha 2 affected cell proliferation, migration, and apoptosis via regulating the dimethylation of eukaryotic translation elongation factor 1 alpha at lysine 55 in acute myeloid leukemia. **Methods:** The expressions of eukaryotic translation elongation factor 1 alpha 2 and dimethylation of eukaryotic translation elongation factor 1 alpha at lysine 55 in acute myeloid leukemia cell lines and human normal bone marrow mononuclear cells (as control) were assessed. Control CRISPR-Cas9 lentivirus, eukaryotic translation elongation factor 1 alpha 2 knockout CRISPR-Cas9 lentivirus, vector plasmid, eukaryotic translation elongation factor 1 alpha 2 wild type overexpression plasmid, and eukaryotic translation elongation factor 1 alpha 2 with a K55R substitution overexpression plasmid were transfected into AML-193 and Kasumi-1 cells combined or alone, and were accordingly divided into 4 groups (Sgcontrol + vector group, SgeEF1A2 + vector group, SgeEF1A2 + eEF1A2$_{WT}$ group, and SgeEFIA2 + eEF1A2$_{K55R}$ group). **Results:** Eukaryotic translation elongation factor 1 alpha 2 and dimethylation of eukaryotic translation elongation factor 1 alpha at lysine 55 expressions were higher in AML-193, Kasumi-1, and KG-1 cell lines compared to the control. In AML-193 and Kasumi-1 cells, the knockout and compensated experiments revealed that eukaryotic translation elongation factor 1 alpha 2 promoted cell proliferation and migration but repressed apoptosis. Additionally, the knockout of eukaryotic translation elongation factor 1 alpha 2 decreased dimethylation of eukaryotic translation elongation factor 1 alpha at lysine 55 expression, meanwhile, eukaryotic translation elongation factor 1 alpha 2 wild type overexpression enhanced while eukaryotic translation elongation factor 1 alpha 2 with a K55R substitution overexpression did not influence the dimethylation of eukaryotic translation elongation factor 1 alpha at lysine 55 expression. Furthermore, eukaryotic translation elongation factor 1 alpha 2 wild type overexpression promoted cell proliferation, enhanced migration, and decreased apoptosis, but eukaryotic translation elongation factor 1 alpha 2 with a K55R substitution overexpression did not influence these cellular functions in AML-193 and Kasumi-1 cells, suggesting the implication of dimethylation of eukaryotic translation elongation factor 1 alpha at lysine 55 in eukaryotic translation elongation factor 1 alpha 2 mediated oncogenesis of acute myeloid leukemia. **Conclusion:** Eukaryotic translation elongation factor 1 alpha 2 and its dimethylated product may serve as therapeutic targets, and these findings may provide support for exploring novel strategies in acute myeloid leukemia treatment.

Keywords

acute myeloid leukemia, eukaryotic translation elongation factor 1 alpha, dimethylation of eEF1A at lysine 55, cell proliferation, cell migration

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Abbreviations

AML, acute myeloid leukemia; cDNA, complementary DNA; eEF1A, eukaryotic translation elongation factor 1 alpha; $eEF1A2_{WT}$, eEF1A2 wild type; eEF1AK55me2, dimethylation of eEF1A at lysine 55; FBS, fetal bovine serum; mRNA, messenger RNA; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; sgRNA, single guide RNA; CCK-8, Cell Counting Kit-8; AV/PI, Annexin V/propidium iodide; eEFs, elongation factors; $eEF1A2_{K55R}$, eEF1A2 harboring a K55R substitution

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Introduction

Acute myeloid leukemia (AML) is a malignant hematopoietic system disease characterized by abnormal proliferation of undifferentiated and nonfunctional leukemic blasts in the bone marrow, and it results in approximately 20% of all hematologic malignancy related deaths.[1] Despite the response rate for AML after initial chemotherapy increases owing to treatment progresses these years, the high relapse rate (20%-70%) and unsatisfactory 5-year survival (less than 30%) still make AML a challenging problem.[2-4] Taking into account the recent advancements in biomolecule expression profiling, exploring molecular networks is an appropriate and timely step to uncover mechanisms of AML.

Translation is the key event involved in the synthesis of protein, and translation factors are the crucial participators in this process.[5,6] As one of the well-investigated translation factors, eukaryotic translation elongation factor 1 alpha (eEF1A) is a GTP-binding protein that interacts with amino-acylated transfer RNA and recruits them to ribosome during the protein translation.[7] Previous data show that eEF1A participates in many cellular biological processes (such as protein translation infidelity as well as cytoskeleton alterations) and various pathological processes (such as immunodeficiency as well as neural defects).[6,8] In accordance with these plenty of functions, eEF1A is highly posttranslationally modified, including methylation.[9] Methylation of eEF1A likewise modulates the eEF1A-mediated biology processes, and it may occur at multiple lysine residues, which is the addition of 1 (me1), 2 (me2), or 3 (me3) methyl groups to a lysine side chain.[10,11] Although eEF1A methylation has been discovered for decades, little is known about its function in disease pathology.[12] Recently, one study reveals that the dimethylation of eEF1A at lysine 55 (eEF1AK55me2), which is a high stoichiometry species, is found overexpressed and predictive for patients' poor outcomes in pancreatic cancer and lung cancer.[13] These findings indicate that eEF1A methylation, especially eEF1AK55me2, may be involved in the cancer progression.

As one of the 2 isoforms of eEF1A (eEF1A1 and eEF1A2), eEF1A2 has been reported to function as an oncoprotein through multiple mechanisms: for instance, promoting cell migration and invasion via upregulating matrix metalloproteinase 9 and activating Akt in pancreatic cancer cells and enhancing proliferation but inhibiting apoptosis through downregulating apoptosis protein caspase 3 and downregulating BAX in prostate cancer cells.[14-16] For hematological malignancies, eEF1A2 expression is also increased in multiple myeloma cell lines than that in normal control cells, and targeting eEF1A2 exhibits antiproliferative effect in myeloid leukemia cell line.[17,18] These previous data imply that eEF1A2 may play an oncogenic role in several malignancies, particular in hematological malignancies.

Considering eEF1A2 might act as a tumor promoter in hematological malignancies, and eEF1A methylation (including eEF1AK55me2) could participate in eEF1A2-mediated regulation to promote cancer progression (based on previous studies), we hypothesized that eEF1A2 might also enhance cell growth and migration via modulating eEF1AK55me2 in AML, however, no relative evidence was observed. Thus, we conducted this study to investigate whether eEF1A2 affected the cell proliferation, apoptosis, and migration via modulating eEF1AK55me2 in AML cell lines.

Materials and Methods

Cell Culture

Human AML cell lines including AML-193, OCI-AML-3, Kasumi-1, and KG-1 were all purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany). The AML-193 cells were grown in the Iscove modified Dulbecco's medium (Gibco, Grand Island, New York) that contains 10% fetal bovine serum (FBS) (Gibco, Grand Island, New York). The OCI-AML-3 cells were cultured in α minimum essential medium (Gibco, Grand Island, New York) that contains 20% FBS (Gibco, Grand Island, New York). The KG-1 and Kasumi-1 cells were maintained in Roswell Park Memorial Institute-1640 medium (Gibco, Grand Island, New York) containing 10% FBS (Gibco, Grand Island, New York). A humidified atmosphere with 5% CO_2 (at 37°C) was provided for all the cells. Human normal bone marrow mononuclear cells (served as control) were isolated from 4 healthy donors (control 1, 2, 3, 4) after approval of ethics committee of our hospital and collection of the written informed consent. The eEF1A2 messenger RNA (mRNA) and protein expressions in AML cells and in control were determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and Western blot. Also, eEF1AK55me2 expression was determined by Western blot.

EEF1A2 KnockOut Cells

To knockout endogenous eEF1A2 in cells, CRISPR-Cas9 system was performed. Briefly, virus particles were produced by cotransfection of 293 T cells (ATCC) with the pCMV-VSV-G

(Addgene, Watertown, Massachusetts), pCMV-dR8.2 dvpr (Addgene, Manassas, Virginia), and modified lentiCRISPR v2 expressing single guide RNA (sgRNA, Watertown, Massachusetts) for 48 hours. After virus were collected, AML-193 cells and Kasumi-1 cells were infected with virus and then selected with puromycin (Sigma, Saint Louis, Missouri). After infection, the cells were termed as Sgcontrol cells and SgeEF1A2 cells. The control sgRNA was: 5′-GACTGGCGGAGCGTGCTATC-3′; and the eEF1A2 knockout sgRNA was: 5′-CTAGCCGCCACTCACGTTGG-3′.

Plasmid Transfection

Overexpression of eEF1A2 plasmid was constructed with pcDNA3.1 vector (GenePharma, China): The pcDNA3.1 vector cloned with eEF1A2 wild type complementary DNA (cDNA) or eEF1A2 mutant cDNA (where a lysine [K] 55 to arginine [R] change was involved) was named $eEF1A2_{WT}$ plasmid or $eEF1A2_{K55R}$ plasmid, respectively; the pcDNA3.1 vector cloned with nonsense DNA fragment was named vector plasmid. After being constructed, plasmids were transfected into Sgcontrol cells and SgeEF1A2 cells. Sgcontrol cells transfected with vector plasmid were named as Sgcontrol + vector cells. The SgeEF1A2 cells transfected with vector plasmid, $eEF1A2_{WT}$ plasmid, and $eEF1A2_{K55R}$ plasmid were named as SgeEF1A2 + vector, SgeEF1A2 + $eEF1A2_{WT}$, and SgeEFIA2 + $eEF1A2_{K55R}$ cells, respectively. After transfection, the expression of eEF1A2 was determined by RT-qPCR and Western blot at 48 hours. The expression of eEF1AK55me2 was assessed by Western blot at 48 hours. Cell proliferation was determined by Cell Counting Kit-8 (CCK-8) assay 0, 24, 48, 72, and 96 hours. Cell apoptosis was detected by Annexin V/propidium iodide (AV/PI) assay at 72 hours. Cell migration was measured by Transwell assay at 72 hours.

RT-qPCR

After cells were collected, PureZOL RNA isolation reagent (Bio-rad, Hercules, California) was used to isolate total RNA. Then, 1 μg total RNA was applied for reverse transcription in a 20 μL reaction of PrimeScript RT reagent Kit (Takara, Japan). At last, TB Green Fast qPCR Mix (Takara, Japan) was applied for quantitative polymerase chain reaction (qPCR) according to the protocol. Results of qPCR was calculated by $2^{-\Delta\Delta Ct}$ formula, and GAPDH was applied as the internal reference for eEF1A2. In detail, the calculation was performed as follows: (1) qPCR was performed in triplicate, and the average values of eEF1A2 Ct and GAPDH Ct in every sample were determined, respectively; (2) calculations of $\triangle Ct$ ($Ct_{avg.eEF1A2} - Ct_{avg.GAPDH}$) were presented in every sample, which was shown as $\triangle Ct_{(sample)}$; (3) the median of $\triangle Ct$ in control was referred as the calibrator, which was shown as $\triangle Ct_{(calibrator)}$; (4) $\triangle\triangle Ct = \triangle Ct_{(sample)} - \triangle Ct_{(calibrator)}$; (5) the relative expression of eEF1A2 was proceeded via calculating $2^{-\triangle\triangle Ct}$. Primers were presented in Table 1.

Table 1. Primers Applied in RT-qPCR.

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
eEF1A2	CTGGAAGGTGGAGCGTAAGGA	GTGCCAATGCCGCCAATCT
GAPDH	GACCACAGTCCATGCCATCAC	ACGCCTGCTTCACCACCTT

Abbreviations: eEF1A2, eukaryotic translation elongation factors 1 alpha 2; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

Table 2. Antibodies Applied in Western Blot.

Antibody	Company	Dilution
Primary antibody		
Anti-eEF1AK55me2 antibody (custom antibody)	ABclonal Biotechnology (China)	1:10 000
Anti-eEF1A2 antibody (ab194441)	Abcam (United Kingdom)	1:1000
Anti-GAPDH antibody (ab125247)	Abcam (United Kingdom)	1:5000
Secondary antibody		
Goat Antimouse IgG-HRP (ab6721)	Abcam (United Kingdom)	1:10 000

Abbreviations: eEF1A2, eukaryotic translation elongation factors 1 alpha 2; eEF1AK55me2, dimethylation of eEF1A at lysine 55; HRP, horseradish peroxidase; IgG, immunoglobulin G.

Western Blot

Total protein was extracted with radioimmunoprecipitation assay buffer (Sigma, Saint Louis, Missouri) containing a protease inhibitor cocktail. After being quantified by a Pierce BCA Protein Assay Kit (Thermo, Waltham, Massachusetts), 20 μg protein was loaded into 4% to 12% Bis-Tris Gels (Thermo, Waltham, Massachusetts) followed by separation. Subsequently, the protein was transferred to polyvinylidene fluoride membrane (Millipore, Germany), and the membrane was blocked with 5% skim milk (Beyotime, China). Thereafter, the membrane was incubated with primary antibodies at 4°C overnight, followed by incubated with secondary antibody at 37°C. After incubation, the membrane was illuminated with Novex-ECL Chemiluminescent Substrate Reagent Kit (Invitrogen, Carlsbad, California). Finally, the images were taken and quantified by Image J (NIH, Carlsbad, California). Antibodies applied are shown in Table 2.

CCK-8 Assay

Cells were grown in 96-well plate. At the beginning of CCK-8, culture medium of cells was replaced with the medium that contains 10 μL CCK-8 reagent (APE_Xbio, Houston, Texas) and 100 μL FBS free medium. Then, the cells were cultured at 37°C for 2 hours. Optical density value was detected by a microplate reader (BioTek, Winooski, Vermont), and it represented the cell proliferation ability.

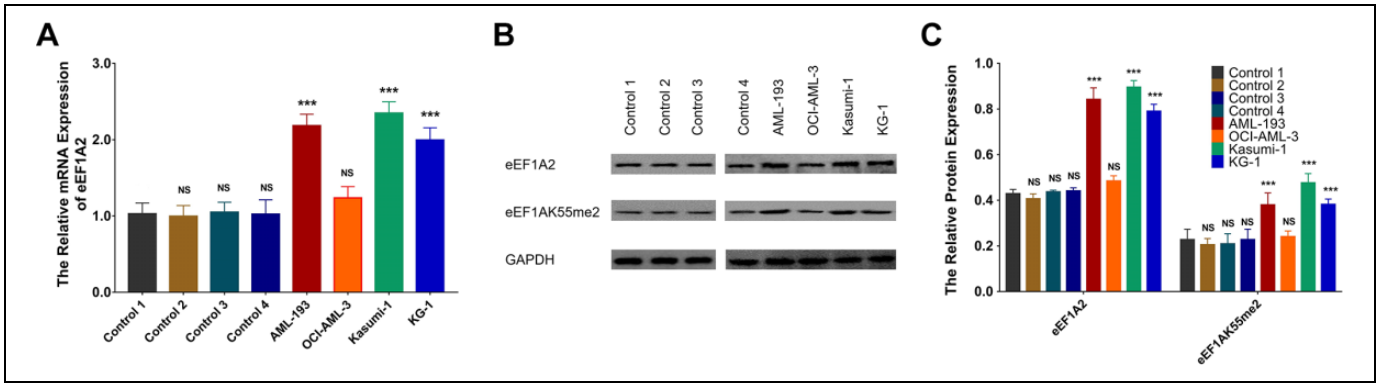

Figure 1. Expressions of eEF1A2 and eEF1AK55me2 in AML cell lines. eEF1A2 mRNA expression (A), eEF1A2 protein expression and eEF1AK55me2 expression (B and C) in AML-93, OCI-AML-3, Kasumi-1, KG-1, and control cells (detection of eEF1A2 and eEF1AK55me2 expressions among various control samples was not performed in the same time, thus the protein bands of control 1 to 3 and control 4 samples were exhibited separately). AML indicates acute myeloid leukemia; eEF1A2, eukaryotic translation elongation factor 1 alpha 2; eEF1AK55me2, dimethylation of eukaryotic translation elongation factor 1 alpha at lysine 55; mRNA, messenger RNA.

AV/PI Assay

The AV/PI assay was performed according to the manufacturers' instruction of Annexin V-FITC Apoptosis Detection Kit (R&D, Minneapolis, Minnesota). Briefly, cells were harvested and resuspended in phosphate buffer saline. After being added with 5 μL AV and 5 μL PI, cell suspension was incubated in dark at 37°C for 15 minutes. Then, CytoFLEX flow cytometer (Beckman Coulter, Fullerton, California) was used to detect the cells, and the apoptosis rate was evaluated by FlowJo 7.6 (FlowJo, Franklin lakes, New Jersey).

Transwell Assay

Briefly, cells in FBS-free culture medium were plated into upper side of transwell chamber (Corning, Corning, New York), and the lower chamber was filled with culture medium containing 10% FBS (Gibco, Grand Island, New York). At 24 hours, the lower chamber medium was collected, and cells (migrated cells) in the medium were calculated with a CytoFLEX flow cytometer (Beckman Coulter, Fullerton, California).

Statistical Analysis

Statistical analysis and graph drawn were carried out by GraphPad Prism 7.02 software (GraphPad Software Inc, San Diego, California). Data were presented as mean and standard deviation. Difference among groups was determined by one-way analysis of variance followed by Dunnett multiple comparisons test. Difference between 2 groups was determined by unpaired *t* test. $P < .05$ was considered significance.

Results

Expressions of eEF1A2 and eEF1AK55me2 in AML Cell Lines and Control Cell Line

The eEF1A2 mRNA (Figure 1A), eEF1A2 protein (Figure 1B and C), and eEF1AK55me2 (Figure 1B and C) expressions in control 2 cells, control 3 cells, and control 4 cells were all similar with those in control 1 cells (all $P > .05$), indicating that they had stable levels among control samples. For eEF1A2 mRNA (Figure 1A) or protein (Figure 1B and C) expressions, they were increased in AML-193, Kasumi-1, and KG-1 cell lines compared to control 1 cells (all $P < .001$), while were similar between OCI-AML-3 cell line and control 1 cells (both $P > .05$). For eEF1AK55me2, its expression was elevated in AML-193 ($P < .001$), Kasumi-1 ($P < .001$), and KG-1 ($P < .001$) cell lines compared to control 1 cells (Figure 1B and C), while was similar between OCI-AML-3 cell line and control 1 cells ($P > .05$). Since the numerically 2 highest eEF1A2 and eEF1AK55me2 expressions were observed in AML-193 cells and Kasumi-1 cells, we chose these 2 cell lines for the subsequent knockout and compensated experiments.

Expressions of eEF1A2 and eEF1AK55me2 After Transfection

In order to further explore the functions of eEF1A2 and eEF1AK55me2 in AML cell lines, we transfected $eEF1A2_{WT}$ overexpression plasmid or $eEF1A2_{K55R}$ overexpression plasmid separately to the eEF1A2 knockout AML-193 cells and eEF1A2 knockout Kasumi-1 cells. In AML-193 cells, eEF1A2 mRNA (Figure 2A) and protein expressions (Figure 2B and C) were decreased in SgeEF1A2 + vector group compared to Sgcontrol + vector group (both $P < .001$), and they were elevated in SgeEF1A2 + $eEF1A2_{WT}$ group and SgeEFIA2 + $eEF1A2_{K55R}$ group compared to SgeEF1A2 + vector group (all $P < .001$), while they were similar between SgeEFIA2 + $eEF1A2_{K55R}$ group and SgeEF1A2 + $eEF1A2_{WT}$ group (both $P > .05$). As to eEF1AK55me2 (Figure 2B and C), its expression was lower in SgeEF1A2 + vector group compared to Sgcontrol + vector group ($P < .01$), and it was raised in SgeEF1A2 + $eEF1A2_{WT}$ group compared to SgeEFIA2 + $eEF1A2_{K55R}$ group ($P < .001$) and SgeEF1A2 + vector group ($P < .001$), but it was similar between SgeEFIA2 +

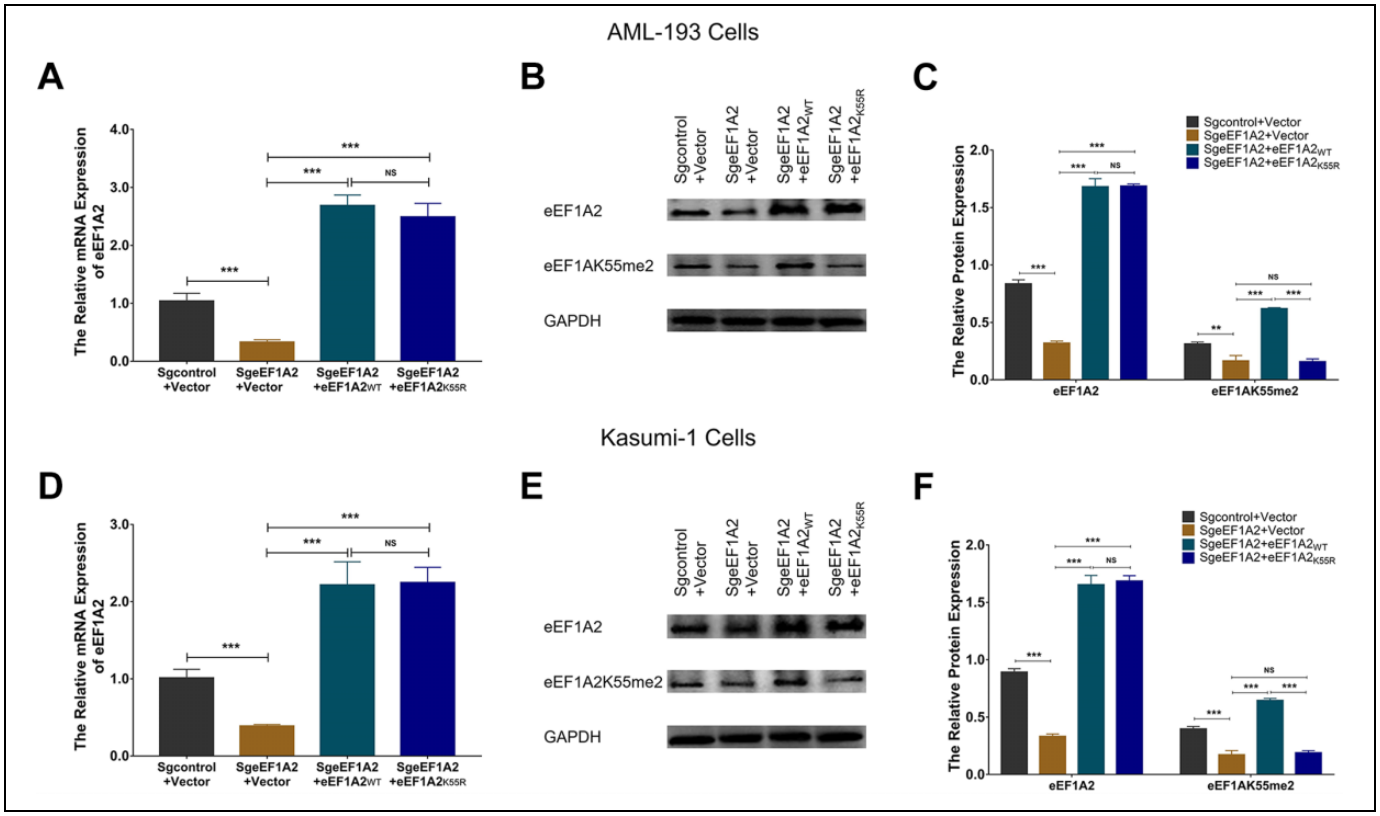

Figure 2. Expressions of eEF1A2 and eEF1AK55me2 after eEF1A2 knockout and eEF1A2 overexpression compensation in AML cell lines. eEF1A2 mRNA expression (A), eEF1A2 protein expression and eEF1AK55me2 expression (B and C) in Sgcontrol + vector, SgeEF1A2 + vector, SgeEF1A2 + eEF1A2$_{WT}$, and SgeEF1A2 + eEF1A2$_{K55R}$ groups in AML-193 cells. eEF1A2 mRNA expression (D), eEF1A2 protein expression and eEF1AK55me2 expression (E, F) in Sgcontrol + vector, SgeEF1A2 + vector, SgeEF1A2 + eEF1A2$_{WT}$, and SgeEF1A2 + eEF1A2$_{K55R}$ groups in Kasumi-1 cells. AML indicates acute myeloid leukemia; eEF1A2, eukaryotic translation elongation factor 1 alpha 2; eEF1AK55me2, dimethylation of eukaryotic translation elongation factor 1 alpha at lysine 55; eEF1A2$_{WT}$, eEF1A2 wild type; eEF1A2$_{K55R}$, eEF1A2 with lysine 55 transferred to arginine; mRNA, messenger RNA.

eEF1A2$_{K55R}$ group and SgeEF1A2 + vector group ($P > .05$). In Kasumi-1 cells, eEF1A2 (Figure 2D-F) and eEF1AK55me2 (Figure 2E and F) expressions exhibited the similar trends among Sgcontrol + vector, SgeEF1A2 + vector, SgeEF1A2 + eEF1A2$_{WT}$, and SgeEFIA2+eEF1A2$_{K55R}$ groups with those in AML-193 cells. These data suggested that the eEF1A2 knockout and eEF1A2 overexpression compensated AML cells were successfully established; more importantly, AML cells with similar eEF1A2 expression but dysregulated eEF1AK55me2 expression were successfully established, which could be applied for the further experiments to assess the effect of eEF1AK55me2 in AML cells. Besides, the proof that eEF1A2 positively regulated eEF1AK55me2 in AML cells could be observed in these data.

Effect of eEF1A2 and eEF1AK55me2 on Cell Proliferation

In AML-193 cells, cell proliferation was reduced in SgeEF1A2 + vector group compared to Sgcontrol + vector group at 72 hours ($P < .05$) and 96 hours ($P < .01$), while it was increased in SgeEF1A2 + eEF1A2$_{WT}$ group compared to SgeEF1A2 + vector group at 24 hours ($P < .05$), 48 hours ($P < .01$), 72 hours ($P < .01$), and 96 hours ($P < .01$), suggesting that eEF1A2 promoted proliferation in AML-193 cells (Figure 3A). Besides, cell proliferation was decreased in SgeEFIA2 + eEF1A2$_{K55R}$ group compared to SgeEF1A2 + eEF1A2$_{WT}$ group at 48 hours ($P < .05$), 72 hours ($P < .01$), and 96 hours ($P < .01$), while it was similar between SgeEFIA2 + eEF1A2$_{K55R}$ group and SgeEF1A2 + vector group (all $P > .05$). In Kasumi-1 cells (Figure 3B), cell proliferation showed the similar trends among Sgcontrol + vector, SgeEF1A2 + vector, SgeEF1A2 + eEF1A2$_{WT}$, and SgeEFIA2+eEF1A2$_{K55R}$ groups with those in AML-193 cells. Combined all these data together, we found that eEF1A2 promoted cell proliferation via modulating eEF1AK55me2 in AML cell lines.

Effect of eEF1A2 and eEF1AK55me2 on Cell Apoptosis

In AML-193 cells, cell apoptosis rate was elevated in SgeEF1A2 + vector group compared to Sgcontrol + vector group ($P < .001$), but it was reduced in SgeEF1A2 + eEF1A2$_{WT}$ group compared to SgeEF1A2 + vector group

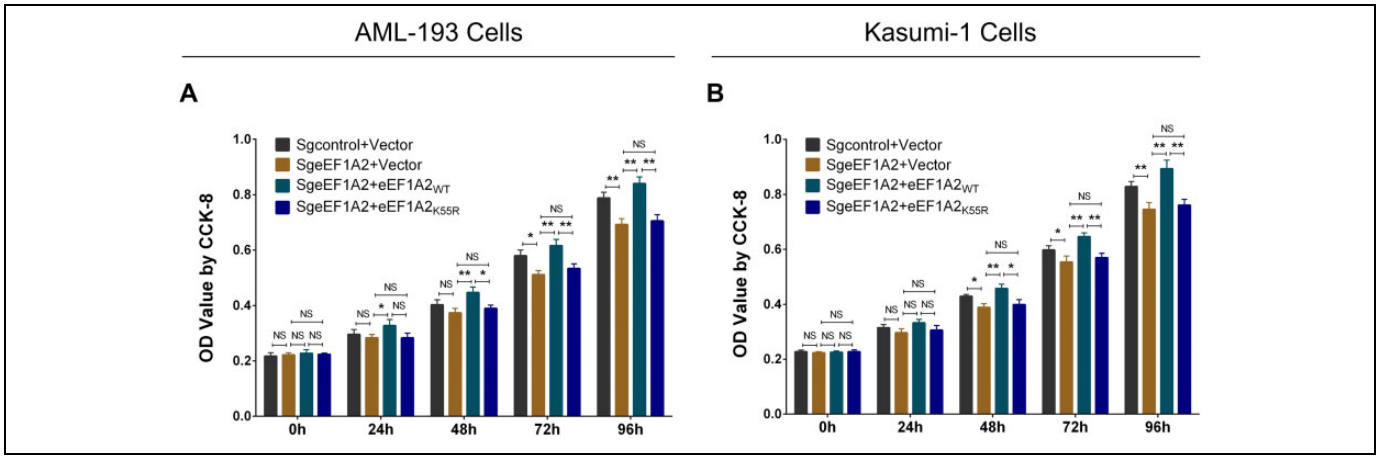

Figure 3. Cell proliferation after eEF1A2 knockout and eEF1A2 overexpression compensation in AML cell lines. Cell proliferation in Sgcontrol + vector, SgeEF1A2 + vector, SgeEF1A2 + eEF1A2$_{WT}$, and SgeEF1A2+eEF1A2$_{K55R}$ groups in AML-193 cells (A) and Kasumi-1 cells (B). eEF1A2$_{WT}$, eEF1A2 wild type; eEF1A2$_{K55R}$, eEF1A2 with lysine 55 transferred to arginine. AML indicates acute myeloid leukemia; eEF1A2, eukaryotic translation elongation factor 1 alpha 2; eEF1A2$_{WT}$, eEF1A2 wild type; eEF1A2$_{K55R}$, eEF1A2 with lysine 55 transferred to arginine.

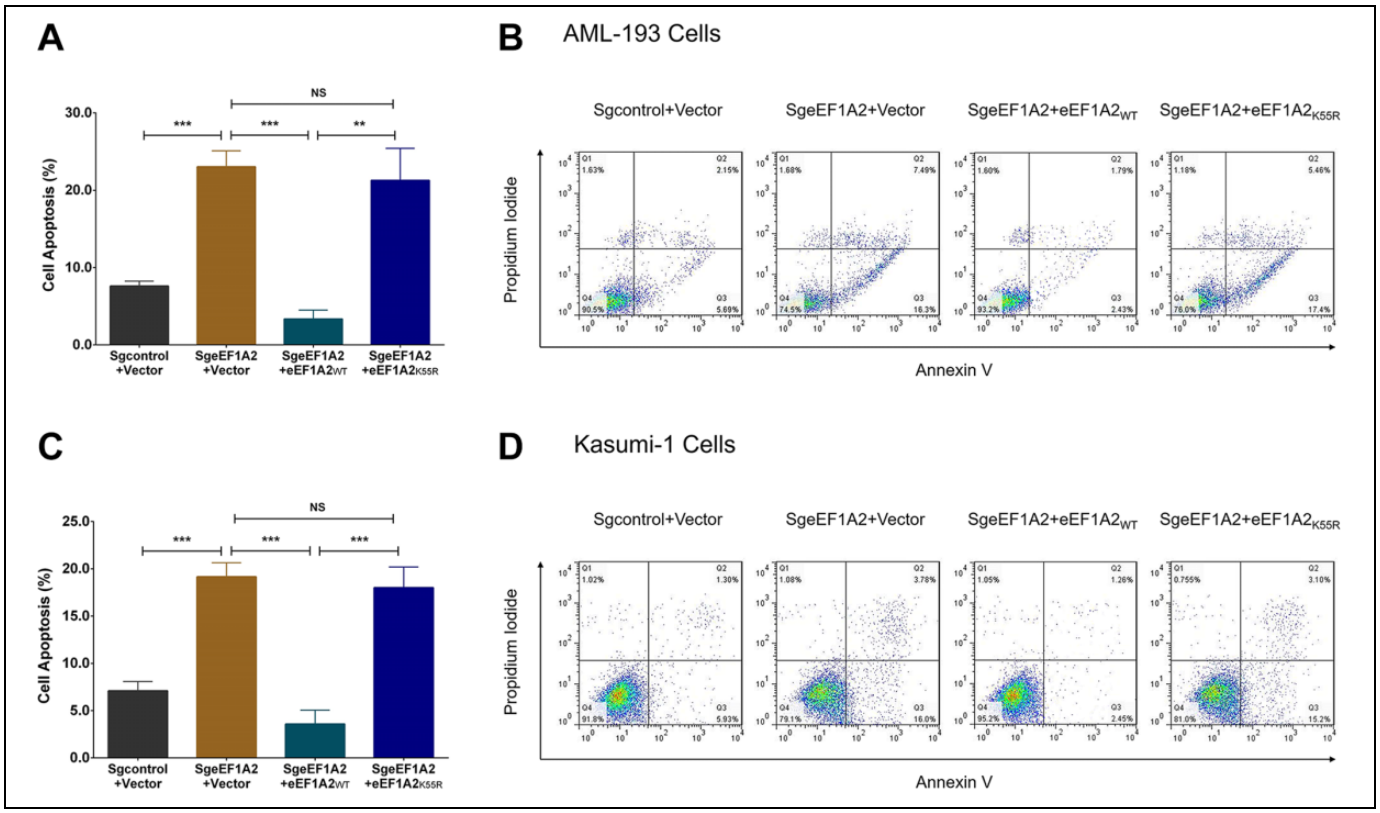

Figure 4. Cell apoptosis after eEF1A2 knockout and eEF1A2 overexpression compensation in AML cell lines. Cell apoptosis in Sgcontrol + vector, SgeEF1A2 + vector, SgeEF1A2 + eEF1A2$_{WT}$, and SgeEF1A2 + eEF1A2$_{K55R}$ groups in AML-193 cells (A and B) and Kasumi-1 cells (C and D). AML indicates acute myeloid leukemia; eEF1A2, eukaryotic translation elongation factor 1 alpha 2; eEF1A2$_{WT}$, eEF1A2 wild type; eEF1A2$_{K55R}$, eEF1A2 with lysine 55 transferred to arginine.

($P < .001$), indicating that eEF1A2 repressed apoptosis in AML-193 cells (Figure 4A and B). Moreover, cell apoptosis rate was increased in SgeEFIA2 + eEF1A2$_{K55R}$ group compared to SgeEF1A2 + eEF1A2$_{WT}$ group ($P < .01$), but it was similar between SgeEFIA2 + eEF1A2$_{K55R}$ group and SgeEF1A2 + vector group ($P > .05$). In Kasumi-1 cells (Figure 4C and D), cell apoptosis rate presented with the similar trends among Sgcontrol + vector, SgeEF1A2 + vector, SgeEF1A2 + eEF1A2$_{WT}$, and SgeEFIA2 + eEF1A2$_{K55R}$ groups with those in AML-193 cells. Taken together, these data

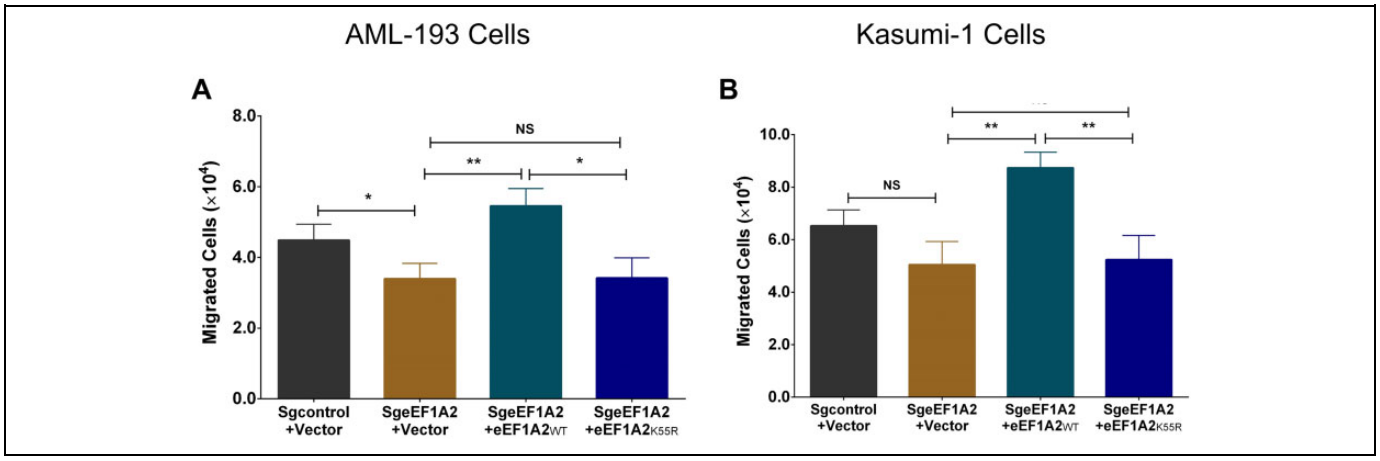

Figure 5. Cell migration after eEF1A2 knockout and eEF1A2 overexpression compensation in AML cell lines. Cell migration in Sgcontrol + vector, SgeEF1A2 + vector, SgeEF1A2 + $eEF1A2_{WT}$, and SgeEF1A2 + $eEF1A2_{K55R}$ groups in AML-193 cells (A) and Kasumi-1 cells (B). AML indicates acute myeloid leukemia; eEF1A2, eukaryotic translation elongation factor 1 alpha 2; $eEF1A2_{WT}$, eEF1A2 wild type; $eEF1A2_{K55R}$, eEF1A2 with lysine 55 transferred to arginine.

indicated that eEF1A2 inhibited cell apoptosis through regulating eEF1AK55me2 in AML cell lines.

Effect of eEF1A2 and eEF1AK55me2 on Cell Migration

In AML-193 cells, the number of migrated cells in SgeEF1A2 + vector group was less than that in Sgcontrol + vector group ($P < .05$), but it was increased in SgeEF1A2 + $eEF1A2_{WT}$ group compared to SgeEF1A2 + vector group ($P < .01$), indicating that eEF1A2 enhanced AML-193 cell migration (Figure 5A). Moreover, the number of migrated cells was reduced in SgeEFIA2 + $eEF1A2_{K55R}$ group compared to SgeEF1A2 + $eEF1A2_{WT}$ group ($P < .05$), while was similar between SgeEFIA2 + $eEF1A2_{K55R}$ group and SgeEF1A2 + vector group ($P > .05$). In Kasumi-1 cells (Figure 5B), cell migration showed the similar trends among Sgcontrol + vector, SgeEF1A2 + vector, SgeEF1A2 + $eEF1A2_{WT}$, and SgeEFIA2 + $eEF1A2_{K55R}$ groups with those in AML-193 cells. These above-mentioned data suggested that eEF1A2 regulated eEF1AK55me2 to promote cell migration in AML cell lines.

Discussion

Our data indicated that (1) eEF1A2 was overexpressed in AML cell lines compared to control cells, and it promoted cell proliferation and migration but decreased apoptosis in AML cell lines; (2) after eEF1A2 knockout, eEF1AK55me2 restored the regulation of eEF1A2 on cell proliferation, apoptosis, and migration in AML cells.

The process of eukaryotic protein synthesis consists of 3 major phases, including the initiation, elongation, and termination.[5,19] For the elongation, eukaryotic translation elongation factors (eEFs) are key elements involved in the translation process of protein, moreover, eEF1A complex is one of the well-investigated eEFs, which may function as oncoproteins via modulating cellular functions of cancer cells.[16,20,21] For example, eEF1A1 downregulation suppresses cell proliferation but decreases cyclin D1, which further affects cell cycle and enhance apoptosis in hepatocellular carcinoma cells.[20] Besides, eEF1A1 overexpression specifically represses p53-, p73- and chemotherapy-induced apoptosis, thereby leading to chemoresistance in cervical carcinoma cells.[21] Additionally, eEF1A2 downregulation results in a great reduction of proliferation, increase of apoptosis rate, and elevates apoptosis marker (caspase 3 and BAX) expressions in prostate cancer cells.[16] Furthermore, eEF1A2 downregulation inhibits cell migration and invasion in pancreatic cancer cells.[15] And an interesting study discloses that eEF1A2 increases cell migration and invasion in a PI3K- and Akt-dependent manner in breast cancer cells.[7] As to hematological malignancies, eEF1A2 is also found overexpressed in multiple myeloma cell lines, and targeting eEF1A2 shows antiproliferative effect on myelogenous leukemia cells.[17,18] These data reveal that eEF1A members, particularly eEF1A2, may facilitate the tumor genesis and progression via promoting cancer cell proliferation, enhancing invasion, and inhibiting apoptosis in malignant diseases, including hematologic malignancies. Therefore, we hypothesized eEF1A2 might also take part in the pathology of AML, however, no relative evidence was reported. Thus, we compared the eEF1A2 expression between AML cell lines and normal bone marrow mononuclear cells, and we observed eEF1A2 was overexpressed in AML cell lines. In addition, we knocked out endogenous eEF1A2 in AML cell lines and then performed eEF1A2 compensation by overexpression plasmids to investigate the effect of eEF1A2 on cell proliferation, apoptosis, and migration in AML cells. We found that eEF1A2 was able to promote cell proliferation and migration but decrease apoptosis in AML cell lines, implying that eEF1A2 might function as an important regulator in the AML mechanism.

With regard to the mechanisms of eEF1A2 in AML, the posttranslational modification of eEF1A2 may play an important role. It is known that eEF1A carries enriched spaces for

posttranslational modifications, and a striking feature of the posttranslational modification landscape of eEF1A is the lysine methylation.[22] Current relevant data mainly focus on the structure, diversity, and dynamic functions of eEF1A lysine methylation. For instance, eEF1A lysine methylation is found to modulate the phenotype-related steps (such as translation rate and replicative lifespan) and maintain the cellular homeostasis (such as ribosome biogenesis and unfolded protein response)[23-25]; whereas, less is known about the role of eEF1A lysine methylation in human malignancies, only one study discloses that eEF1AK55me2 is overexpressed and enhances the proliferation in pancreatic cancer cells.[13] Considering eEF1A2 might play a carcinogenic role in hematological malignancies, and eEF1AK55me2 took part in eEF1A2-mediated regulation to enhance cancer progression, we hypothesized that eEF1A2 might modulate eEF1AK55me2 to influence malignant cell functions in AML cells, while no relative literature was found. To validate our assumption, in our study, we compared the eEF1AK55me2 expression between AML cell lines and normal bone marrow mononuclear cells. We found that eEF1AK55me2 was overexpressed in AML cell lines. Furthermore, after eEF1A2 knockout and eEF1A2 overexpression compensation were performed in AML cells, we constructed AML cells that similarly expressed eEF1A2 but differentially expressed eEF1AK55me2, which could be applied to investigate whether eEF1A2 regulated AML cellular functions via affecting eEF1AK55me2. The results showed that only compensation with the wild-type eEF1A2, but not eEF1A2 harboring a K55R substitution ($eEF1A2_{K55R}$), could restore the regulation of eEF1A2 on cell proliferation, apoptosis, and migration in AML cells. Our data implied that eEF1A2 regulated eEF1AK55me2 to further promote the proliferation, migration, but decrease apoptosis in AML cell lines. The following reasons might explain these findings: (1) eEF1AK55me2 might increase the GTPase activity, which boosted the translation elongation, and thereby enhanced protein synthesis that were essential for cell proliferation, thus, eEF1AK55me2 upregulation promoted the cell proliferation in AML cell lines[13]; (2) eEF1AK55me2 might bind to unphosphorylated STAT1 to repress cell apoptosis, thus eEF1AK55me2 upregulation reduced the apoptosis in AML cell lines[26]; and (3) eEF1AK55me2 might activate the actin remodeling and Akt to enhance the Akt-dependent cell migration, therefore eEF1AK55me2 upregulation enhanced cell migration in AML cell lines.[6] Our results might provide indications for the further explorations of AML mechanism.

In conclusion, eEF1A2 promotes cell proliferation and migration, but inhibits apoptosis via inducing eEF1AK55me2 in AML. Our findings indicate that eEF1A2 and its dimethylated product may serve as therapeutic targets and thereby providing support for exploring novel strategies in AML treatment.

Authors' Note

Shan Xiao and Yanping Wang contributed equally to this work. Our study did not require an ethical board approval because it did not contain human or animal trials.

Declaration of Conflicting Interests

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References

1. Kreitz J, Schonfeld C, Seibert M, et al. Metabolic plasticity of acute myeloid leukemia. *Cells*. 2019;8(8):2.
2. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med*. 2015;373(12):1136-1152.
3. Estey E, Dohner H. Acute myeloid leukaemia. *Lancet*. 2006; 368(9550):1894-1907.
4. Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 2010;115(3):453-474.
5. Thornton S, Anand N, Purcell D, Lee J. Not just for housekeeping: protein initiation and elongation factors in cell growth and tumorigenesis. *J Mol Med (Berl)*. 2003;81(9):536-548.
6. Abbas W, Kumar A, Herbein G. The eEF1A Proteins: at the crossroads of oncogenesis, apoptosis, and viral infections. *Front Oncol*. 2015;5(1):75.
7. Amiri A, Noei F, Jeganathan S, Kulkarni G, Pinke DE, Lee JM. eEF1A2 activates Akt and stimulates Akt-dependent actin remodeling, invasion and migration. *Oncogene*. 2007;26(21): 3027-3040.
8. Lamberti A, Caraglia M, Longo O, Marra M, Abbruzzese A, Arcari P. The translation elongation factor 1A in tumorigenesis, signal transduction and apoptosis: review article. *Amino Acids*. 2004;26(4):443-448.
9. Hamey JJ, Wilkins MR. Methylation of elongation factor 1A: where, who, and why? *Trends Biochem Sci*. 2018;43(3): 211-223.
10. Helen CM. Hospital programs emphasize community and patient good will. *Hosp Top*. 1968;46(9):35-36.
11. Robichaud N, Sonenberg N, Ruggero D, Schneider RJ. Translational control in cancer. *Cold Spring Harb Perspect Biol*. 2019; 11(7):8.
12. Hiatt WR, Garcia R, Merrick WC, Sypherd PS. Methylation of elongation factor 1 alpha from the fungus Mucor. *Proc Natl Acad Sci U S A*. 1982;79(11):3433-3437.
13. Liu S, Hausmann S, Carlson SM, et al. METTL13 Methylation of eEF1A increases translational output to promote tumorigenesis. *Cell*. 2019;176(3):491-504. e421.

14. Kim J, Namkung W, Yoon JS, et al. The role of translation elongation factor eEF1A in intracellular alkalinization-induced tumor cell growth. *Lab Invest*. 2009;89(8):867-874.
15. Xu C, Hu DM, Zhu Q. EEF1A2 promotes cell migration, invasion and metastasis in pancreatic cancer by upregulating MMP-9 expression through Akt activation. *Clin Exp Metastasis*. 2013; 30(7):933-944.
16. Sun Y, Du C, Wang B, Zhang Y, Liu X, Ren G. Up-regulation of eEF1A2 promotes proliferation and inhibits apoptosis in prostate cancer. *Biochem Biophys Res Commun*. 2014;450(1):1-6.
17. Li Z, Qi CF, Shin DM, et al. Eef1a2 promotes cell growth, inhibits apoptosis and activates JAK/STAT and AKT signaling in mouse plasmacytomas. *PLoS One*. 2010;5(5):e10755.
18. Losada A, Munoz-Alonso MJ, Garcia C, et al. Translation elongation factor eEF1A2 is a novel anticancer target for the marine natural product plitidepsin. *Sci Rep*. 2016;6:35100.
19. Sasikumar AN, Perez WB, Kinzy TG. The many roles of the eukaryotic elongation factor 1 complex. *Wiley Interdiscip Rev RNA*. 2012;3(4):543-555.
20. Huang J, Zheng C, Shao J, Chen L, Liu X, Shao J. Overexpression of eEF1A1 regulates G1-phase progression to promote HCC proliferation through the STAT1-cyclin D1 pathway. *Biochem Biophys Res Commun*. 2017;494(3-4):542-549.
21. Blanch A, Robinson F, Watson IR, Cheng LS, Irwin MS. Eukaryotic translation elongation factor 1-alpha 1 inhibits p53 and p73 dependent apoptosis and chemotherapy sensitivity. *PLoS One*. 2013;8(6):e66436.
22. Jakobsson ME, Malecki J, Falnes PO. Regulation of eukaryotic elongation factor 1 alpha (eEF1A) by dynamic lysine methylation. *RNA Biol*. 2018;15(3):314-319.
23. Pan KZ, Palter JE, Rogers AN, et al. Inhibition of mRNA translation extends lifespan in Caenorhabditis elegans. *Aging Cell*. 2007;6(1):111-119.
24. Hansen M, Taubert S, Crawford D, Libina N, Lee SJ, Kenyon C. Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. *Aging Cell*. 2007; 6(1):95-110.
25. Malecki J, Aileni VK, Ho AYY, et al. The novel lysine specific methyltransferase METTL21B affects mRNA translation through inducible and dynamic methylation of Lys-165 in human eukaryotic elongation factor 1 alpha (eEF1A). *Nucleic Acids Res*. 2017; 45(8):4370-4389.
26. Yao K, Chen Q, Wu Y, Liu F, Chen X, Zhang Y. Unphosphorylated STAT1 represses apoptosis in macrophages during Mycobacteriumtuberculosis infection. *J Cell Sci*. 2017;130(10): 1740-1751.