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ORIGINAL RESEARCH

Expression and Clinical Value of Eukaryotic Translation Elongation Factor IAI (EEFIAI) in Diffuse Large B Cell Lymphoma

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¹Institute of Hematology and Oncology, Harbin the First Hospital, Harbin, 150010, People's Republic of China; ²Department of Lymphatic Hematology and Oncology, Jiangxi Cancer Hospital, Nanchang, 330029, People's Republic of China **Background:** The eukaryotic translation elongation factor 1A1 (EEF1A1) participates in protein translation and has been reported to be involved in tumor progression such as hepatocellular carcinoma. Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid malignancy in adults. In the present study, we aimed to detect the expression of EEF1A1 in DLBCL and to analyze its relationship with prognosis.

Methods: We reviewed medical records of DLBCL patients in our hospital and evaluated their expression level of EEF1A1 in tumor tissues using immunohistochemical (IHC) assay. The Chi-square method was used for correlation analysis. The Kaplan–Meier method with Log rank test was used for univariate analysis. Cox proportional hazards model was used for multivariate analysis. Cellular and mice models were introduced to validate its oncogenic role.

Results: EEF1A1 expression in tumor cells was higher in certain DLBCL cases. Patients with higher EEF1A1 expression were more likely to have advanced tumor stage and poorer 5-year overall survival (OS) rates. EEF1A1 expression in tumor cells was an independent risk predictor for OS (P < 0.05). Cellular assays demonstrated that EEF1A1-shRNA significantly inhibited lymphoma cell proliferation. The study of xenografts further verified the effect of EEF1A1-shRNA on suppressing tumor growth in vivo.

Conclusion: EEF1A1 positivity predicts short survival in DLBCL patients. For patients with higher EEF1A1 expression, more strategy such as anti-EEF1A1 antibody treatment should be developed.

Keywords: diffuse large B-cell lymphoma, prognosis, EEF1A1, proliferation

Introduction

Characterized by growing tumor mass in single or multiple lymph nodes as well as extranodal sites,¹ diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL), accounting for 30% of NHL cases worldwide.² There are approximately 15,000 newly diagnosed DLBCL cases in the United States,³ and about 25,000 new cases in China each year.⁴ Although more than 80% of DLBCL patients may achieve a complete remission under immuno-chemotherapy, a significant minority (20% to 25%) of these patients will suffer with relapse.⁵ The increasing incidence and unsatisfied prognosis are attracting more and more attentions. Therefore, understanding the oncogenesis mechanism and progression regulators of DLBCL are urgently needed for prognosis prediction and therapy development.

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Eukaryotic elongation factor-1 (EF1) contains four subunits, EF1 α , EF1 β , EF1 γ , and EF1 δ .⁶ Eukaryotic translation elongation factor 1 alpha 1 (EEF1A1) is a predominant isoform of the EEF1 α complex, which participates in tRNA delivery by promoting the GTPdependent binding of aminoacyl-tRNA to the A-site of ribosomes.⁷ EEF1A1 is ubiquitously expressed in many tissues due to its critical function in protein synthesis. Besides protein synthesis, EEF1A1 plays roles in cytoskeleton organization, cell apoptosis, stabilizing RNAs, etc.⁸ Ditzel et al identified EEF1A1 as an autoantibody in about 66% of the patients with Felty syndrome, an disorder characterized by autoimmune diseases such as rheumatoid arthritis and splenomegaly.⁹ Dysregulated EEF1A1 has been identified in many diseases. For example, hyperexpression of EEF1A1 was reported in spinal and bulbar muscular atrophy and amyotrophic lateral sclerosis.¹⁰ In contrast, a hypoexpression of EEF1A1 is observed during myocardial infarction and may be involved in cardiovascular disorders.¹¹

Of note, the potential role of EEF1A1 in tumor progression has been reported in certain malignancies. For example, Grassi et al reported a higher mRNA level of EEF1A1 in hepatocellular carcinoma cell lines compared to normal liver cells.¹² A similar conclusion was obtained based on the protein level of EEF1A1 in clinical resected liver cancer tissues.¹³ Consistently, EEF1A1 was highly expressed in clear cell renal cell carcinomas (RCC) and associated with poor clinical outcomes. Knockdown of EEF1A1 impaired the viability of RCC cells.¹⁴

In contrast, EEF1A1 seems to play tumor suppressing roles in colon cancer and breast cancer. High expression of EEF1A1 was reported to predict a favorable overall survival of colon adenocarcinoma patients.¹⁵ According to the data by Lin et al, EEF1A1 mRNA levels are reduced in clinical breast ductal carcinoma specimens. Underexpression of EEF1A1 mRNA predicts poor prognosis for estrogen receptor-positive cancer patients.¹⁶ However, another study by Li et al indicated overexpressing EEF1A1 can promote breast cancer cell proliferation and invasion.¹⁷

Here we investigated the expression profile and clinical significance of EEF1A1 in DLBCL for the first time. Accordingly, high EEF1A1 is closely correlated with unfavorable overall survival of DLBCL patients. The pro-oncogenic role of EEF1A1 in DLBCL was further validated by in vitro and in vivo assays.

Methods Online Database

The transcription profiling of EEF1A1 gene expression in DLBCL and normal lymph nodes were performed using the Gene Expression Profiling Interactive Analysis (GEPIA) database (<u>http://gepia.cancer-pku.cn/index.html</u>). We compared the mRNA level, which was presented as transcripts per million (TPM), from The Cancer Genome Atlas (TCGA, <u>https://www.cancer.gov/tcga</u>) tumors vs TCGA normal + The Genotype-Tissue Expression (GTEx, <u>https://gtexportal.org/</u>) normal datasets.¹⁸ The prognostic value of EEF1A1-TPM was also extracted from GEPIA database and compared by Log rank test.

Patients and Clinical Specimens

A total of 138 DLBCL tissues were acquired from Harbin The First Hospital. The median age at the time of diagnosis was 61 years and median follow-up time was 47 months (range, 6-119 months). All tissue specimens were formalin-fixed and paraffin-embedded. All diagnoses were based on pathological test. The characteristics of enrolled patients are listed in Table 1. Among all cases, 56 were female and 82 were male. Eighty-nine cases exhibited B symptoms, while the other 49 cases without B symptoms. Seventy-two cases were staged as Ann Arbor Stage I-II, and 66 cases with stage III-IV. As for the Eastern Cooperative Oncology Group Performance Status (ECOG PS), 90 cases were scored as 0 or 1, while the other 48 cases with scores larger than 1. The serum lactate dehydrogenase (LDH) level at the time of diagnosis was also retrieved. Accordingly, 69 cases exhibited normal LDH level, while the other 69 cases with elevated LDH level. The number of extra nodal involvement (ENI) was less than 2 in 91 patients, and larger than or equal to 2 in 47 patients. Twenty-two cases showed tumors with 10 cm in diameter or larger (Bulky tumor), and the other 115 cases with smaller tumor size. International Prognostic Index (IPI) was less than 3 in 93 cases, while scored as 3-5 in the other 45 cases. Finally, we assessed the Cell-of-Origin (COO) subtyping and found that 61 cases showed germinal center B cell (GCB) subtype, while the other 77 cases showed non-GCB subtype.

Immunohistochemistry (IHC)

IHC was conducted to explore the EEF1A protein expression level in clinical tissue samples. Briefly, the tissue

Variables	Cases	EEFIA	P value	
	(n=138)	Low (n=55)	High (n=83)	
Sex				
Female	56	20 (35.7%)	36 (64.3%)	0.412
Male	82	35 (42.7%)	47 (57.3%)	
Age				
≤60 years	66	27 (40.9%)	39 (59.1%)	0.809
>60 years	72	28 (38.9%)	44 (61.1%)	
B symptoms				
Absence	89	33 (37.1%)	56 (62.9%)	0.369
Presence	49	22 (44.9%)	27 (55.1%)	
Ann Arbor				
Stage				
-	72	39 (54.2%)	33 (45.8%)	<0.001*
III–IV	66	16 (24.2%)	50 (75.8%)	
ECOG PS				
0-1	90	42 (46,7%)	48 (53.3%)	0.025*
≥ 2	48	13 (27.1%)	35 (72.9%)	0.010
Serum I DH				
level				
Normal	69	39 (56.5%)	30 (43.5%)	<0.001*
Elevated	69	16 (23.2%)	53 (76.8%)	
FNI				
<2	91	35 (38.5%)	56 (61.5%)	0.642
≥2	47	20 (42.6%)	27 (57.4%)	
Bulky tumor	1			
No	115	43 (37.4%)	72 (62.6%)	0.186
Yes	23	12 (52.2%)	11 (47.8%)	
IPI	1			
0-2	93	48 (51.6%)	45 (48.4%)	<0.001*
3–5	45	7 (15.6%)	38 (84.4%)	
соо	1			
GCB	61	26 (42.6%)	35 (57.4%)	0.554
Non-GCB	77	29 (37 7%)	48 (62 3%)	

 Table I Characteristics of DLBCL Patients and Their

 Correlations with EEFIAI Level

Note: *P<0.05 by Chi-square test.

Abbreviations: DLBCL, diffuse large B cell lymphoma; EEFIAI, eukaryotic translation elongation factor IAI; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase; ENI, extra nodal involvement; IPI, International Prognostic Index; COO, Cell-of-Origin; GCB, germinal center B cell.

sections were deparaffinized, rehydrated, and then incubated with 3% hydrogen peroxide (H2O2). Antigen retrieval was achieved by using ethylenediaminetetraacetic acid (EDTA) buffer. The tissue sections were blocked with 5% bovine serum (BSA) and then probed with anti-EEF1A1 (1:300, #sc-21758, Santa Cruz Biotechnology) at 4 °C overnight. Secondary antibody was then added and incubated. The immunoreactivity was finally detected by using the diaminobenzidine (DAB) staining reagents according to the manufacturer's instructions.¹⁹

The IHC results were next scored regarding both staining intensity and the percentage of positive stained cells.²⁰ Staining intensity score was given as negative staining: 1; weak staining: 2; moderate staining: 3; and strong staining: 4. Percentage of positive cells was scored as 0-25%: 1; 26-50%: 2; 51-75%: 3; and >75%: 4. The immunoreactivity score was obtained by multiplying the two scores above, ranging 0-16. Then, a receiver operating characteristic (ROC) curve was plotted to define a cut-off value for distinguishing low-EEF1A1 expression and high-EEF1A1 expression.

Cell Culture and Transfection

Human DLBCL cell lines (OCI-LY1, OCI-LY3, OCI-LY7, OCI-LY10) were acquired from the American Tissue Culture Collection (ATCC). All cells were routinely maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS). The cells were cultured in a 37 °C incubator with 5% carbon dioxide.

The shRNA targeting EEF1A1 was constructed in lentivirus vectors as described before.¹⁴ The coding region for EEF1A1 was inserted into puro-CMV vectors by Gene Pharma (Shanghai, China) for overexpressing EEF1A1. Lentivirus transduction was conducted according to the standard procedures.²¹ Briefly, 293T cells were firstly seeded into 10 cm dishes and cultured for 24 hours till 80% confluence. Then, 2 µg of lentivirus vector plasmids containing shRNAs were transfected into 293T cells using lipofectamine transfection reagent (Invitrogen, Carlsbad, CA) to generate lentivirus. Cell culture supernatants of 293T cells containing the lentivirus were collected at different time points (day 1, day 2) and pooled. Then, OCI-LY7 cells were transduced with appropriated amount of lentivirus collected above. Forty-eight hours posttransduction, cells were subjected to Western blot and functional assays.

Western Blotting (WB)

WB was used to test protein expression levels as previously described.^{22,23} Briefly, RIPA buffer was used to lyse cells and extract proteins (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. After quantifying the protein concentration and denaturing, same amount of protein samples was separated by SDS-PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis). The proteins were then transferred onto the PVDF (polyvinylidene fluoride) membrane, followed by incubation with primary antibody (1:1000 dilution) and goat antimouse secondary antibody (1:10,000 dilution). The protein level was finally visualized by testing the immunoreactivity of the PVDF membrane.

Cell Viability by CCK-8

The cell viability was detected by cell counting kit-8 (CCK-8) assay as we described previously.²⁵ Briefly, stable transfected cells and control cells were seeded into a 96-well plate at a density of 2×10^3 cells/well. After cultured at 37 °C with 5% CO₂ for designated time points (8, 24, 48, 72, and 96 hours). CCK-8 reagent was then added to each well and incubated for another 2 hours. The absorbance was recorded at 490 nm on a plate reader.²⁴ Each experiment was repeated three times.

Subcutaneous Tumor Growth in Nude Mice

Male BALB/c nude mice (4-weeks old, 15–18g) were purchased from Animal Center of Harbin The First Hospital. The mice were housed under constant temperature of 22–25°C and humidity of 55%, with standard diet and tap water. Stable transfected cells were subcutaneously injected into the nude mice (n=14, respectively). Tumor size was measured using vernier calipers every 5 days. After 4 weeks, the mice were sacrificed, and the tumors were separated and weighed.²⁶

Statistics

Clinical correlation data was analyzed using Chi-square test. Cellular data were presented as mean \pm standard deviation (SD). Data were analyzed using SPSS software and Image J software. Statistical significance was tested using Student's *t*-test or one-way ANOVA test. P-value <0.05 was considered statistically significant.

Ethics

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Harbin The First Hospital. Written informed consents were obtained from all participants.

The animal study was approved and supervised by the Animal Center of Harbin The First Hospital and carried

out in accordance with the UK Animals (Scientific Procedures) Act, 1986.

Results

The Transcriptional Level of *EEFIA1* is Elevated in DLBCL and Correlated with Poor Prognosis

By extracting the transcriptional information of *EEF1A1* in DLBCL and normal lymph nodes from the GEPIA online server, we found that *EEF1A1* possessed more transcripts in DLBCL than that in normal lymph nodes (Figure 1A and B). We next generate the survival curve using Kaplan–Meier method to evaluate whether *EEF1A1*-mRNA level has any prognostic significance. As a result, higher *EEF1A1* transcripts indicate poorer overall survival of DLBCL cases (Figure 1C), although the case number is limited.

Protein Expression of EEFIAI in DLBCL and Its Correlation with Clinicopathological Characteristics

Considering the possibility of difference between mRNA and protein levels, we next set to explore the protein expression pattern of EEF1A1 in DLBCL tissues collected from our hospital (n=138). According to the IHC data, EEF1A1 exhibited predominant cytoplasm localization and slight nucleus staining. Importantly, diverse immunoreactivities among DLBCL tissues were observed (Figure 2A and B), highlighting the high heterogeneity of DLBCL. To better assess the correlations between EEF1A1 level and DLBCL progression, we defined a high-EEF1A group and a low-EEF1A group based on the IHC staining results using a ROC curve (Figure 2C). Therefore, 53 cases were subgrouped into the low-EEF1A1 group, while the other 83 cases as high-EEF1A1 expression.

As shown in Table 1, patients with more advanced tumor stages showed high EEF1A1 protein expression than those with earlier stages (P<0.001). Consistently, ECOG PS and IPI both exerted positive correlation with EEF1A1 expression (P=0.025 and P<0.001, respectively). In addition, the serum LDH level at the time of diagnosis, an important clinical predictor for DLBCL prognosis, tend to be elevated in patients with higher EEF1A1 expression (P<0.001). In contrast, no statistically significant correlation was observed between EEF1A1 with patients'



Figure 1 The transcriptional level of *EEFIA1* is elevated in DLBCL and correlated with poor prognosis. (**A** and **B**) The transcriptional level of *EEFIA1* was retrieved from GEPIA online database, showing significant enriched transcripts in DLBCLs than those in nontumorous normal lymph nodes. (**C**) The prognostic role of *EEFIA1* transcripts on predicting DLBCL overall survival was evaluated by Kaplan–Meier method.



Figure 2 Protein expression level of EEFIA1 in DLBCL. Representative high (A) and low (B) protein immunostaining of EEFIA1 of DLBCL tissues. The ROC curve was plotted to determine a cut-off value to distinguish high- and low-EEFIA1 expression (C). * indicates P<0.05.

B symptoms, ENI, tumor size, nor cell origin (all P>0.05). The clinical associations imply that EEF1A1 may participate in DLBCL progression.

High EEFIAI Protein Expression Serves as an Independent Prognostic Factor

The follow-up information of our enrolled cohort was next analyzed. By the end of follow-up, 65 cases dead. The five-year overall survival rate was 56.5% with a median survival time as 80 months (Figure 3A). Prognostic values of conventional clinicopathological characteristics as well as EEF1A1 protein level were next evaluated by Kaplan– Meier analyses (Table 2, Figure 3). Accordingly, the survival time of patients with stage III–IV was 51.6 ± 5.3 months, while was 88.3 ± 5.2 months for stage I–II patients (Figure 3B, P<0.001). Higher ECOG PS also exhibited unfavorable effect on patients' survival. The 5-year overall survival rate of cases with ECOG PS ≥ 2 was only 22.1%, significantly lower than those with ECOG PS < 2 (70.5%; Figure 3C, P<0.001). Similarly, an elevated serum LDH level indicated a shorter overall survival time (57.6 \pm 5.9 months) compared to patients with normal LDH level (84.6 \pm 5.0 months, Figure 3D). Additionally, patients with extra nodal involvement (P=0.019) or non-GCB subtype (P=0.002) also exhibited



Figure 3 Overall survival analyses of DLBCL patients. The overall survival curves were plotted by Kaplan–Meier method for the entire cohort (A), or based on tumor stage (B), ECOG PS (C), serum LDH level (D), ENI (E), IPI (F), COO (G), EEFIAI expression level (H), respectively. *Indicates P<0.05 by Log rank test.

Table 2 Kaplan-Meier Overall Survival Analyses

Variables	Cases (n=138)	OS Months (Mean ± S.D.)	5-Year OS (%)	P value
Sex				
Female	56	71.0 ± 6.1	55.5%	0.810
Male	82	73.1 ± 5.5	57.0%	
Age				
≤60 years	66	77.9 ± 5.5	64.2%	0.229
>60 years	72	66.3 ± 5.9	49.0%	
B symptoms				
Absence	89	78.0 ± 5.0	60.6%	0.064
Presence	49	61.0 ± 6.6	49.1%	
Ann Arbor Stage				
I–II	72	88.3 ± 5.2	73.4%	<0.001*
III–IV	66	51.6 ± 5.3	34.7%	
ECOG PS				
0-1	90	83.9 ± 4.6	70.5%	<0.001*
≥ 2	48	45.2 ± 6.8	22.1%	
Serum LDH level				
Normal	69	84.6 ± 5.0	68.1%	0.002*
Elevated	69	57.6 ± 5.9	44.3%	
ENI				
<2	91	78.3 ± 5.0	61.8%	0.019*
≥2	47	57.2 ± 6.5	43.7%	
Bulky tumor				
No	115	72.8 ± 4.5	57.7%	0.886
Yes	23	67.1 ± 8.8	50.6%	
IPI				
0–2	93	81.0 ± 4.6	66.2%	0.001*
3–5	45	52.2 ± 7.5	33.7%	
соо				
GCB	61	86.2 ± 5.7	69.9%	0.002*
Non-GCB	77	59.6 ± 5.4	45.2%	
EEFIAI protein level				
Low	55	91.3 ± 5.3	74.7%	<0.001*
High	83	56.2 ± 5.2	43.1%	

Note: *P<0.05 by Log rank test.

Abbreviations: DLBCL, diffuse large B cell lymphoma; EEFIAI, eukaryotic translation elongation factor IAI; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase; ENI, extra nodal involvement; IPI, International Prognostic Index; COO, Cell-of-Origin; GCB, germinal center B cell.

poorer prognosis (Figure 3E and G). Consistent with previous studies, IPI index also showed prognostic significance on predicting patients' survival (Figure 3F, P=0.001). Of note, the mean overall survival time of patients with high EEF1A1 protein expression was only 56.2 ± 5.2 months, leading to a 5-year overall survival rate as 43.1% (Figure 3H). In contrast, patients with lower EEF1A1 levels showed a significantly longer overall survival time (91.3 \pm 5.3 months) and higher 5-year survival rate (74.7%). According to the data in our cohort, patients' age, sex, B symptoms or tumor size exhibited no significant effect on DLBCL prognosis (Supplemental Figure S1).

All significant variables were then subjected into a Cox regression model to conduct the multivariate analysis except IPI score due to its involvement of other subparameters. The

Table 3 Multivariate Analysis

Variables	HR	95% CI	P value
Ann Arbor Stage (III–IV vs I–II)	2.091	1.205-3.628	0.009*
ECOG PS (≥ 2 vs 0–1)	2.373	1.379-4.084	0.002*
Serum LDH (elevated vs normal)	2.008	1.183–3.410	0.010*
ENI (≥ 2 vs <2)	1.535	0.911–2.587	0.108
COO (non-GCB vs GCB)	1.971	1.094–3.552	0.024*
EEFIAI level (high vs low)	2.091	1.205-3.628	0.009*

Note: *P<0.05 by Cox regression test.

Abbreviations: EEFIA1, eukaryotic translation elongation factor IA1; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase; ENI, extra nodal involvement; COO, Cell-of-Origin; GCB, germinal center B cell.

hazard ratio (HR) and 95% confidence interval (CI) are shown in Table 3. The advanced Ann Arbor Stage (HR=2.091, 95% CI 1.205–3.628, P=0.009), higher ECOG PS (HR=2.973, 95% CI 1.379–4.084, P=0.002), elevated serum LDH level (HR=2.008, 95% CI 1.183–3.410, P=0.010), and non-GCB subtype (HR=1.971, 95% CI 1.094–3.552, P=0.024) all independently contributed to unfavorable prognosis of DLBCL. Importantly, higher EEF1A1 was also identified as a novel independent risk factor for a poor prognosis of DLBCL patients (HR=2.091, 95% CI 1.205–3.628, P=0.009).

EEFIAI Promotes DLBCL Growth Both in vitro and in vivo

Considering the clinical significance of EEF1A1 in DLBCL progression and prognostic prediction, we next tested its cellular role. Western blotting data demonstrated detectable but different endogenous expression levels of EEF1A1 in various DLBCL cell lines (Figure 4A). We therefore selected OCI-LY7 cell line for further experiments due to its moderate expression level. The OCI-LY7 cells stably expressed exogenous EEF1A1 or stably knocked down of EEF1A1 were established and tested by immunoblotting (Figure 4B). By assessing cell viability with CCK-8 assay, we found that overexpressing EEF1A1 significantly promoted cell proliferation, while silencing EEF1A1 exhibited an opposite effect (Figure 4C).

Nude mice were then selected to establish a subcutaneous xenograft model as described in the Method section. By comparing the growth curves of implanted DLBCL, EEF1A1-overexpression group exhibited a most rapid growth speed, while the EEF1A1-shRNA group was the slowest (Figure 4D). Consistent with growth curve, isolated xenografts showed significant differences on both tumor size and tumor weight after resected at day 27 post implantation (Figure 4E and F). Taken together, our data revealed a positive role of EEF1A1 on facilitating DLBCL growth both in vitro and in vivo.

Discussion

Protein synthesis is critical for numerous physiological functions, which can be affected by many factors such as translational initiation, elongation, and termination. Disruption of protein translational steps will result in pathological disorders and diseases.²⁷ As a translational elongation element, deregulation of EEF1A has been reported to be involved in oncogenesis, apoptosis, viral infections, et al.²⁸ Interestingly, both the isoforms of EEF1A, namely the EEF1A1 and EEF1A2, regulate oncogenesis and tumor progression on various aspects.^{29,30} However, the role of EEF1A1 in different cancer types seems distinct.

Here, we firstly demonstrated the upregulated expression of EEF1A1 in DLBCL than in nontumorous lymph nodes on both mRNA and protein levels. Our data also indicated a positive correlation between EEF1A1 and DLBCL progressive characteristics, such as tumor stage and serum LDH level. Furthermore, EEF1A1 was validated as an independent prognostic predictor for DLBCL cases based on our retrospective cohort. Our data are consistent with the pro-oncogenic role of EEF1A1 in hepatocellular carcinoma (HCC) and renal cell carcinoma (RCC). As previously reported, EEF1A1 can promote HCC progression by enhancing cell proliferation without significantly affecting apoptosis,^{12,31} which is closely correlated with STAT1-cyclin D1-dependent cell cycle.32 Nevertheless, EEF1A1 can both promote proliferation and attenuate apoptosis in RCC by modulating activities of ERK and AKT.¹⁴ Similarly, eEF1A1 knockdown resulted in remarkable proliferation inhibition and apoptosis induction of human acute T lymphocytic leukemia cells, which may be mediated by the down-regulation of PI3K/Akt/NF-ĸB and PI3K/Akt/mTOR signaling pathways.³³ EEF1A1 is also believed to regulate the cell apoptosis process in both tumor cells³⁴ and nontumorous cells.^{35–37} Therefore, EEF1A1 is a multifaceted regulator during tumorigenesis and tumor progression.

Of note, the therapeutic value of EEF1A1 has been revealed in chronic lymphocytic leukemia,³⁸ hepatocarcinoma,³⁹ and breast cancer cells.⁴⁰ Our results exhibited the significant effect of silencing EEF1A1 on attenuating DLBCL growth both in vitro and vivo, thus providing experimental evidence for its further investigation



Figure 4 EEFIAI promotes DLBCL growth both in vitro and in vivo. Western blotting was conducted to test the endogenous protein level of EEFIAI in DLBCL cell lines (A). The lentivirus transduction efficiency was tested for the OCI-LY7 cells with shRNA-knockdown and EEFIAI overexpression (B). Cell viability was tested by CCK-8 assay (C). The growth of subcutaneous implanted xenografts was monitored and plotted (D). The excised tumors were photographed (E) and weighted (F). *Indicates P<0.05 by one-way ANOVA test.

as a novel drug target in DLBCL treatment. Our study has several limitations. Firstly, all retrospectively enrolled cases were obtained from a single medical center and may result in regional bias. Secondly, it has been recently reported that EEF1A may exhibit dysregulated GTPase activity during tumorigenesis.⁴¹ However, here we only mapped the total protein expression pattern of EEF1A1 without testing its GTPase activity. More studies focusing on systematically illustrating its functional mechanisms will be necessary for translational medicine.

Conclusions

High EEF1A1 promotes DLBCL growth and is closely correlated with unfavorable overall survival of DLBCL patients.

Data Sharing Statement

The data regarding the current study are available on reasonable request.

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Disclosure

The authors report no conflicts of interest in this work.

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