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Original Research

EEF1A1 is Involved the Regulating Neuroinflammatory Processes in Parkinson's Disease

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Abstract

Background: Studies have reported that the RNA-binding protein Eukaryotic Elongation Factor 1A1 (EEF1A1) is low expressed in the hippocampal region of Alzheimer's disease (AD). In addition, it is related to *PARK2* activity in cells, predicting its importance in neurodegenerative diseases. However, the function of EEF1A1 in Parkinson's disease (PD) is unclear. Our study's primary objective was to knock down EEF1A1 in U251 cells and preliminarily explore the role of EEF1A1 in PD neuroinflammation. **Methods**: To inhibit EEF1A1 from being expressed in U251 cells, siRNA was transfected into those cells. Then, RNA-seq sequencing was used to determine the Differentially Expressed Genes (DEGs) resulting from the EEF1A1 knockdown. Additionally, gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) enrichment analyses were performed to find the biological processes and signaling pathways engaged in the DEGs, as well as to screen for genes associated with neuroinflammatory processes that influence the development of PD. Further Real Time - quantitative Polymerase Chain Reaction (RT-qPCR) validation experiments were performed to confirm the reliability of the sequencing results. Finally, combined with the support of related literature, the molecular mechanism of EEF1A1 in regulating the neuroinflammatory process of PD was initially explored. **Results**: Analysis using the RNA-seq technique showed that EEF1A1 knockdown could significantly upregulate the expression of *IL-6*, *GDF15*, *STC1*, *MT1E*, *GPNMB*, *CCL5*, *MT1X*, *A2M*, and *VIP* genes at the transcriptional level. These nine highly elevated genes were enriched to signaling pathways linked to inflammatory processes, according to an analysis of GO and KEGG enrichment. **Conclusions**: EEF1A1 is involved in the regulating of *IL-6*, *GDF15*, *STC1*, *MT1E*, *GPNMB*, *CCL5*, *MT1X*, *A2M*, and *VIP* genes associated with the neuroinflammatory process of PD. Among them, we found that *GDF15*, *STC1*, *MT1E*, *MT1X*, *GPNMB*, *VIP*, and *A2M* genes were involved in delaying the neuroinflammatory process of PD, while *IL-6* and *CCL5* were involved in exacerbating the neuroinflammatory process, implicating that EEF1A1 may participate in the regulation of the PD neuroinflammation.

Keywords: EEF1A1; Parkinson's disease; neuroinflammation; RNA-seq

1. Introduction

Parkinson's disease (PD) is a commonly occurring neurodegenerative disease. It is characterized by the gradual degeneration of the nigrostriatal dopaminergic pathway, with massive loss of nigrostriatal neurons, exhaustion of dopamine, and abnormal accumulation of Lewy bodies within neurons [1]. PD pathogenesis is involved in a variety of factors, including neuroinflammation, apoptosis, mitochondrial dysfunction, oxidative stress, protein degradation pathway dysfunction, genetics, environment, lifestyle, etc. [2]. Although multiple factors are relevant to the development of PD, the role of neuroinflammatory processes in PD has received increasing attention.

Researchers have reported that microglia, as well as astrocytes are involved in sustained inflammatory responses in neurodegenerative diseases [3,4]. Activation of microglia, an increase in astrocytes, and inflammatory factors are seen in the brain tissue of PD patients [5,6]. An increase in reactive microglia, astrocytes, and death of dopaminergic neurons was caught after a 6-Hydroxydopamine hydrobromide (6-OHDA) injection in the brain's striatum [7,8]. After several years of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injection into experimental mice, an increase in microglia was observed in the central nervous system of mice [9]. The S100b released from astrocytes which is a pro-inflammatory factor that causes increased production of cyclooxygenase-2 (COX-2), nitric oxide (NO), and superoxide radicals in microglia, and directly or indirectly leads to neuronal death can be observed in the substantia-nigra region of the PD patients [10,11]. Activated microglia and astrocytes promote an increase in several inflammatory mediators such as tumor necrosis factor-a (TNF-α), interleukin-6 (IL-6), nitric oxide synthase (NOS2), COX-2, and reactive oxygen species (ROS) [5]. These molecules mediate the efficient presentation of neoantigens to CD4+ T cells through the MHC-II pathway, leading to dopaminergic neuronal death [12]. In addition, these inflammatory factors trigger the ac-

tivation of the microglia, which may cause damage to the substantial nigra-striatal pathway. Increased inflammatory factors such as interleukin-1β (IL-1β), interleukin-2 (IL-2), IL-6, and TNF-α are observed in the brain striatum, serum, and cerebrospinal fluid of PD patients [13]. Pro-inflammatory factors,including IL-1β and TNF-α, induce oxidative stress and neuronal death, especially the death of dopaminergic neurons in PD [14], which suggest the crucial role of neuroinflammation in the pathogenesis of PD.

Eukaryotic elongation factor 1A (EEF1A) is an RNA-binding protein that binds to amyl-tRNA, transfers it to the receptor site of the ribosome during the extension cycle of peptide synthesis, and participates in translation, nuclear export, transcription, and apoptosis [15]. In mammals, EEF1A is divided into EEF1A1 and EEF1A2 [16]. Recent studies have shown that EEF1A1 and Cag A synergistically regulate IL-6 expression by impacting the activation of p-STATS727 in the cell nucleus [17]. Yulan Liu *et al*. [18] found that EEF1A1 may have a significant role in moderating microglia polarization. These reports indicate that EEF1A1 may have an essential role in regulating inflammatory processes. Beckelman *et al*. [19] found that EEF1A expression is reduced in the hippocampus of Alzheimer's disease (AD) patients and that dysregulation of EEF1A and its related signaling pathways may be one of the pathogenic mechanisms of neurodegenerative diseases such as AD. George G *et al*. [20] found that EEF1A1 is associated with PARK2 activity in cells, and is involved in the Parkin ubiquitination-proteasome system (UPS), immune-related biological processes. Vera M *et al*. [21] found that EEF1A1 participates in the entire heat shock response (HSR) process from transcription to translation in mammalian cells. EEF1A1 makes the heat shock response process fast and precise by regulating transcriptional outputs to meet translation requirements. Cells with EEF1A1 deletion expressed severe impairment of HSR and heat resistance, indicating that EEF1A1 may be an essential influencing factor for neurodegeneration and many other diseases associated with the disruption of proteostasis. However, the mechanism of EEF1A1's role in PD is unclear and needs to be further exploration.

Based on the above studies, we knocked down EEF1A1 in U251 cells, performed RNA-seq high-throughput techniques to identify the genes regulated by EEF1A1, and initially explored the association between EEF1A1 and neuroinflammatory processes in PD pathogenesis. We found explicity expressed genes in EEF1A1 knockdown cells, which were involved in the neuroinflammatory process of PD, suggesting that EEF1A1 might affect the regulating of the neuroinflammatory process in PD.

2. Materials and Methods

2.1 Cell Culturing

Cell line: The human glioma cell line U251 was purchased from the Procell (CL-0237; Wuhan, Hubei, China). The U251 cells were certified by STR analysis and tested by the supplier for the absence of mycoplasma contamination. DMEM medium (Dulbecco's modified Eagle's medium) containing 10% fetal bovine serum, 100 µg/mL streptomycin, and 100 U/mL penicillin at 37 °C (CL-0237, Procell Life Sciences Co., Ltd., Wuhan, China) with 5% CO_2 to incubate the cells.

2.2 siRNA Transfection

The three biological experimental groups and the corresponding control groups were established as well as three siRNAs (see Table 1) were synthesized from Gemma (Suzhou, China). The siRNAs was transfected into U251 cells using Lipofectamine™ RNAiMAX transfection reagent (13778030, Invitrogen, Carlsbad, CA, USA) as the experimental group, while the empty plasmid was transfected into U251 cells as the control group. After 48 h of incubation, these transfected cells were collected, and the expression of EEF1A1 was measured by real-time quantitative polymerase chain reaction (RT-qPCR) method to detect the expression of EEF1A1.

2.3 Assessing Knockdown of EEF1A1

The glycerol-3-phosphate dehydrogenase (GAPDH) was used as a control gene for assessing the effects of EEF1A1 knockdown. cDNA synthesis was done by standard procedures, and RT-qPCR was performed on the Bio-Rad S1000 with Hieff™ qPCR SYBR® Green Master Mix (Low Rox Plus; Yeasen, Shanghai, China). The concentration of each transcript was then normalized to GAPDH mRNA levels by the $2^{-\Delta\Delta CT}$ method [22]. Three siRNAs were transfected into cells as the experimental group and empty plasmid was transfected into cells as the control group, and RT-qPCR was performed (Sample information and intra-group consistency and inter-group variability of each sample are shown in **Supplementary Table 1**). RT-qPCR results suggested that EEF1A1 was successfully knocked down at the mRNA level with knockdown efficiencies between 60%–80%. Combining intra-group consistency, inter-group variability, and the appropriate knockdown efficiency, Transfection of siRNA-knockdown sequence 3 cell samples-1, 3, 4 and Cell samples of the control group-1, 3, 4 (NC-1, NC-3, NC-4, Si 3-1, Si 3-3, and Si 3-4) were selected for downstream experiments.

2.4 RNA Extraction and RNA-seq Sequencing

First, TRIZOL(15596-018, Amibion, TX, USA) was used to extract the total RNA, and then, RQ1 DNase (M6101, Promega, Madison, WI, USA) was added to clear the DNA. The quality and quantity of the purified RNA were determined by measuring the absorbance at 260 nm/280 nm (A260/A280) using Nanodrop One (N50 Touch, Implen GmbH, Munich, Germany). RNA integrity was verified by 1.5% agarose gel electrophoresis.

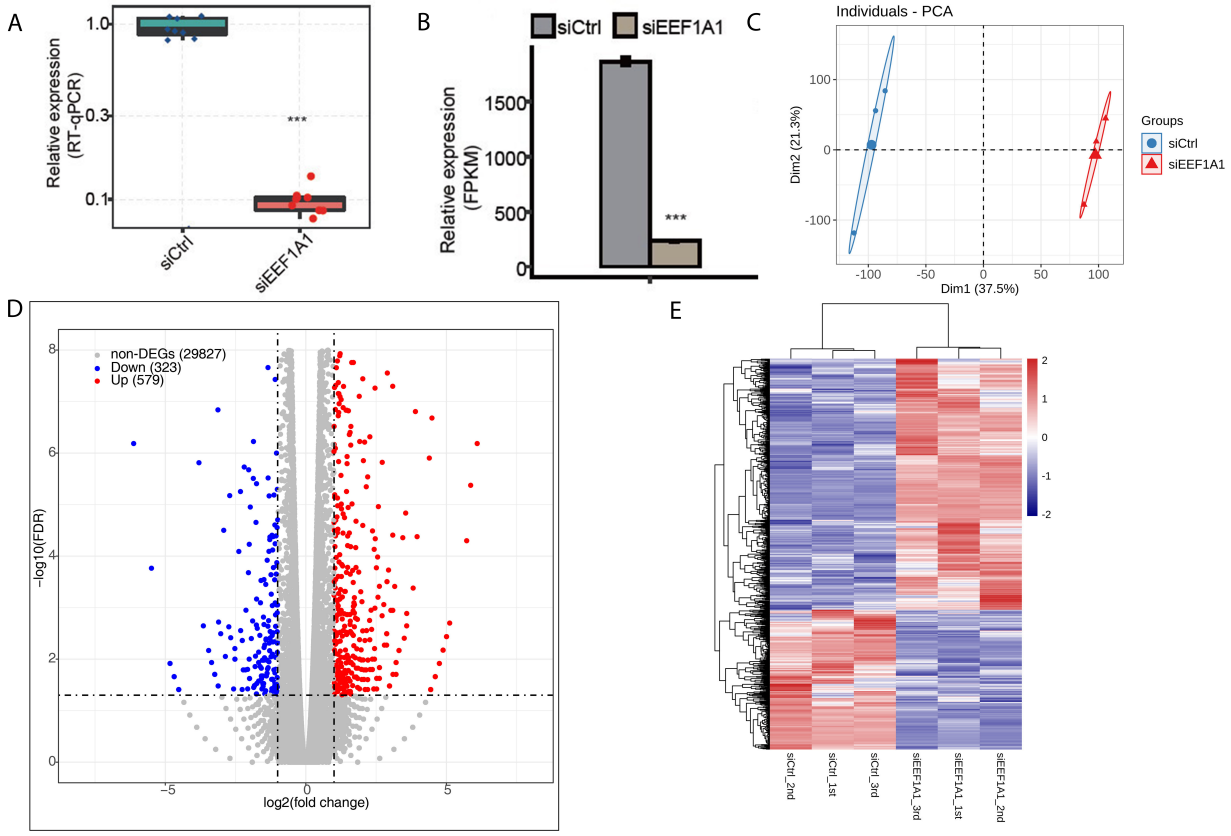

Fig. 1. EEF1A1 regulated differential gene expression in U251 cells. (A) After knocking down EEF1A1 in U251 cells, EEF1A1 expression was detected by qRT-PCR. Error bars represent mean ± SEM. ***$p < 0.001$. (B) EEF1A1 expression quantified by RNA-seq data. Error bars represent mean ± SEM. ***$p < 0.001$. (C) Sample correlation analysis and PCA results demonstrate the overall transcriptome level differences between EEF1A1 knockdown cells and control cells. (D) Genes were significantly expressed after EEF1A1 knockdown, as shown by the volcano map. (E) Hierarchical clustering of DEGs in the experimental and control groups. Columns on the coordinate axes indicate samples, and rows indicate genes. Different colors represent genes with varying changes og fold. Red means up-regulated expression, while blue means down-regulated expression. EEF1A1, Eukaryotic Elongation Factor 1A1; PCA, Principal Component Analysis; qRT-PCR, quantitative real-time polymerase chain reaction; DEGs, Differentially Expressed Genes; FPKM, fragments per kilobase of exon per million fragments mapped.

For each sample, the VAHTS stranded mRNA-seq library preparation kit (NR605-02, Vazyme, China) was used. 1 µg of total RNA was used for RNA-seq library preparation. Polyadenylated mRNA was purified and fragmented, then converted to double-stranded cDNA. After end repair and the addition of single deoxyadenosine A, DNA was added to diluted Roche adapters (KK8726). After purifying the ligated products and subdividing their size to 300–500 bps, the ligated products were amplified, filtered, quantified, and stored at –80 °C. Then, the Illumina NovaSeq6000 system was applied for 150-nt paired-end sequencing.

2.5 Analyzing RNA-seq Data

Firstly, discard raw sequences that contain over 2-N bases. Secondly, adapters and low-quality bases were pruned from the raw sequence reads using the FASTX toolbox (version 0.0.13), and sequences smaller than 16 nt were removed. Finally, valid sequences were aligned to the GRCh38 genome using TopHat2 [23], allowing four mismatches. Uniquely positioned sequences were to be used for gene sequence counting and paired-end fragments per kilobase of exon per million fragments mapped (FPKM) calculations [24].

2.6 Differentially Expressed Genes (DEGs) Analysis

DEGs were selected using the R Bioconductor software package edge R [25]. The false discovery rate (FDR) <0.05, fold change (FC) >2, or <0.5 serves as a test standard for identifying DEGs.

2.7 Functional Enrichment Analysis of DEGs

Gene ontology (GO), kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis was used to predict gene function on KOBAS 2.0 server [26].

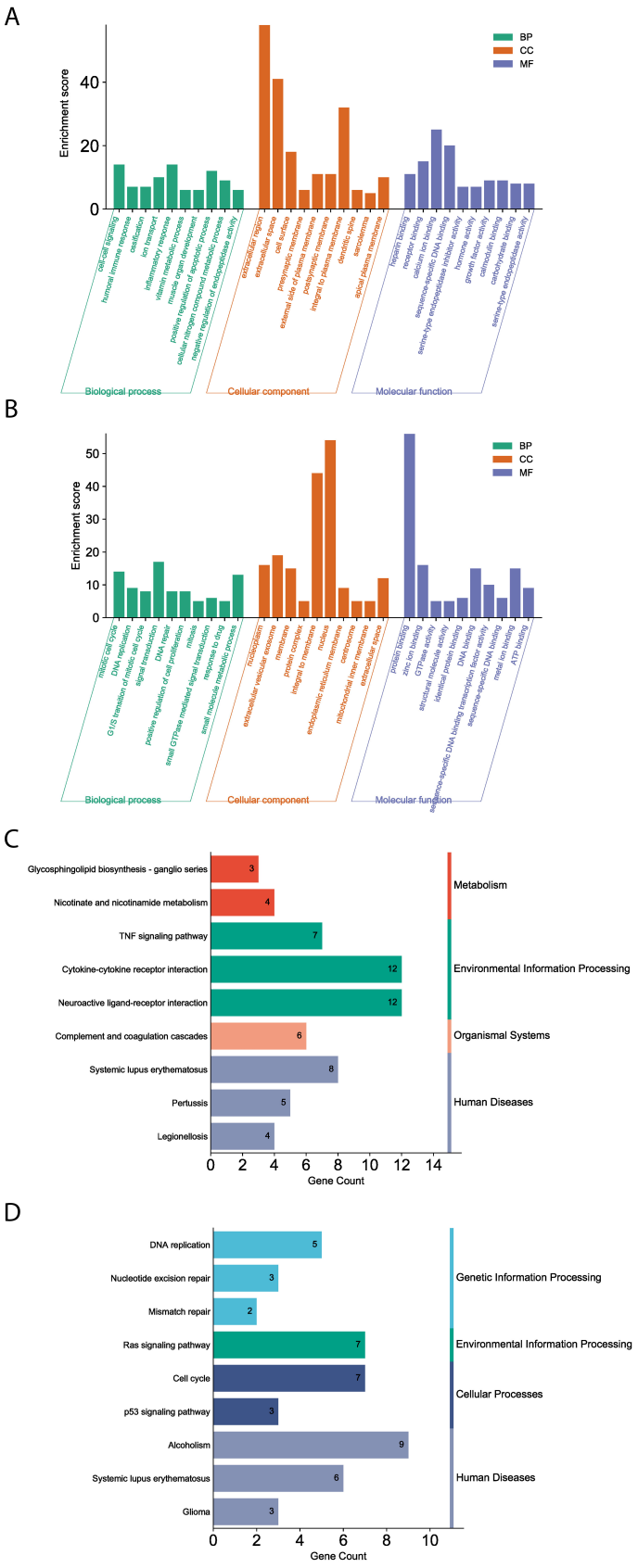

Fig. 2. GO, KEGG results of DEGs. (A,B) The top 10 representatives GO biological processes of up-regulated and down-regulated genes. (C,D) The top 10 representatives of KEGG pathways for up-regulated and down-regulated genes. GO, gene ontology; KEGG, kyoto encyclopedia of genes and genomes.

Table 1. siRNA primer information.

Number	*Gene*	Sense sequence (5′-3′)	Antisense sequence (5′-3′)
siNC	*siNC*	UUCUCCGAACGUCACGUTT	ACGUGACACGUUCGGAGAATT
Si-1	*EEF1A1-Homo-311*	GCAAGUACUAUGUGACUAUTT	AUAGUCACAUAGUACUUGCTT
Si-2	*EEF1A1-Homo-673*	CCAAGUGCUAACAUGCCUUTT	AAGGCAUGUUAGCACUUGGTT
Si-3	*EEF1A1-Homo-884*	CCGGUAUGGUGGUCACCUUTT	AAGGUGACCACCAUACCGGTT

Table 2. Specific gene information related to the inflammatory pathways involved in the up-regulated DEGs in GO analysis.

#term	Corrected *p*-value	Input
Cell-cell signaling	0.001299205	TNFAIP6|BMP3|INHBA|CGA|CCL5|CCR1|LTA|NFE2|CXCL10|GDF15|STC1|STC2|FGFR3|FGF21
Humoral immune response	0.001299205	ST6GAL1|TNFRSF21|IL6|LTA|BST1|LTF|MNX1
Inflammatory response	0.006035552	NGF|SERPINA3|CCL5|MGLL|NLRC4|TNFAIP6|RARRES2|SCN9A|IL6|CCR1|CXCL2|PTX3|CXCL10|CLEC7A
Negative regulation of endopeptidase activity	0.021848981	GSTO2|QPRT|CYP24A1|ACP5|SLC2A3|NMNAT3

DEGs, Differentially Expressed Genes; GO, gene ontology.

Table 3. Specific gene information related to the inflammatory pathways involved in the up-regulated DEGs in KEGG analysis.

#term	Corrected *p*-value	Input
Complement and coagulation cascades	0.517270694	SERPING1|C1S|C2|FGG|PROC|A2M
TNF signaling pathway	0.517270694	CASP7|CXCL2|CCL5|IL18R1|IL6|LTA|CXCL10
Cytokine-cytokine receptor interaction	0.606526412	IL20RB|INHBA|CCL5|LTA|IL18R1|IL6|CCR1|TNFRSF21|CXCL2|INHBE|CXCL10|TNFRSF19

TNF, tumor necrosis factor; KEGG, kyoto encyclopedia of genes and genomes.

Table 4. Significantly up-regulated DEGs after EEF1A1 knockdown.

Gene	Symbol	Log FC	$p < 0.05$
ENSG00000136244.11	*IL-6*	1.486523064	1.25×10^{-45}
ENSG00000130513.6	*GDF15*	1.303437387	4.51×10^{-55}
ENSG00000159167.11	*STC1*	1.117230008	1.22×10^{-40}
ENSG00000169715.14	*MT1E*	1.043478664	3.79×10^{-36}
ENSG00000136235.15	*GPNMB*	2.023675309	1.20×10^{-121}
ENSG00000271503.5	*CCL5*	1.430090424	7.75×10^{-9}
ENSG00000187193.8	*MT1X*	1.207610718	6.32×10^{-44}
ENSG00000175899.14	*A2M*	2.965694647	3.43×10^{-59}
ENSG00000146469.12	*VIP*	3.461931072	9.03×10^{-15}

EEF1A1, Eukaryotic Elongation Factor 1A1.

2.8 RT-qPCR Validation of DEGs

RT-qPCR was performed on DEGs using the SYBR Green PCR kit (11203ES03, Yeasen, Shanghai, China) to confirm the validity of the RNA-seq data. The total RNA remaining from the RNA-seq library preparation was counter-transcribed to cDNA using M-MLV reverse transcriptase (R101-02, Vazyme, Nanjing, China), denatured 40 times at 95 °C for a total of 15 s, annealed, and extended at 60 °C for 1 min. Each sample was PCR amplified in triplicate.

3. Results

3.1 Specifically Up-Regulated Genes were Found in EEF1A1 Knockdown U251 Cells

EEF1A1 expression was measured by RT-qPCR after being knocked down in U251 cells, and it was considerably lower compared to the control group (Fig. 1A). After constructing three biological replicates of RNA-seq samples on experimental and control cells, mass-filtered reads were aligned to the human GRch38 genome. Then, the expression values of genes generated after EEF1A1 knockdown were calculated in FPKM units. We found that the six samples yielded a total of 30,729 expressed genes. (FPKM >0) , the FPKM value of EEF1A1 in the experimental group was greatly reduced (Fig. 1B). When all expressed genes' FPKM values were analyzed using a principal component method, it was discovered that knocking down EEF1A1 was the primary factor affecting gene expression (Fig. 1C). Edge R was used to identify the 902 DEGs regulated by EEF1A1, including 579 up-regulated genes and 323 down-regulated genes, it is presented using a volcano map (Fig. 1D). Based on heat map analysis of DEGs expression patterns in RNA-seq samples, the three experimental groups showed a high degree of concordance in EEF1A1-mediated transcription (Fig. 1E).

3.2 GO, KEGG Enrichment Analysis Results

First, we performed GO enrichment analysis for DEGs, and the results were as follows: The top 10 pathways that highly up-regulated genes were enriched the cell-cell signaling, humoral immune response, ossification, ion transport, inflammatory response, vitamin metabolic process, muscle organ development, positive regulation of the apoptotic process, cellular nitrogen compound metabolic process, and negative regulation of endopeptidase activity (Fig. 2A). The top 10 pathways that significantly down-regulated genes were enriched the mitotic cell cycle, DNA replication, G1/S transition of the mitotic cell cycle, signal transduction, DNA repair, positive regulation of cell proliferation, mitosis, small GTPase mediated signal transduction, response to the drug, and small molecule metabolic processes (Fig. 2B).

The top 10 pathways found to be significantly up-regulated by KEGG analysis were the glycosphingolipid biosynthesis-ganglia series, nicotinate and nicotinamide metabolism, complement and coagulation cascades, systemic lupus erythematosus, TNF signaling pathway, cytokine-cytokine receptor interaction, neuroactive ligand-receptor interaction, pertussis, Legionellosis, and glycosphingolipid biosynthesis-globoid series (Fig. 2C). The top 10 pathways with significant down-regulated gene enrichment were DNA replication, alcoholism, cell cycle, systemic lupus erythematosus, HTLV-I infection, Ras signaling pathway, nucleotide excision repair, p53 signaling pathway, glioma, and mismatch repair (Fig. 2D).

Among them, cell-cell signaling, humoral immune response, inflammatory response, negative regulation of endopeptidase activity, complement and coagulation cascades, TNF signaling pathway, and cytokine-cytokine receptor interactions pathways are associated with inflammatory processes. Significantly up-regulated genes *IL-6*, *CCL5*, *GDF15*, *STC1*, and *A2M* were enriched in these pathways related to the inflammatory processes (Tables 2,3). In addition, We found that EEF1A1 knockdown was followed by a considerable elevated in the expression of gene *MT1X*, and *MT1E* in the perinuclear region, which are involved in the inflammatory process, protects cells from injury caused by free radicals produced by oxidative stress in astrocytes. The expression of *GPNMB*, a gene with anti-inflammatory effects in macrophages and microglia, and *VIP*, a gene with anti-inflammatory and antioxidant properties, was also significantly increased (**Supplementary Table 2**, Table 4).

3.3 The RT-qPCR Validation Results

RT-qPCR was performed to analyze the relative expression trends of *IL-6*, *CCL5*, *GDF15*, *STC1*, *A2M*, *MT1X*, *MT1E*, *GPNMB*, and *VIP* genes in the control and experimental groups, respectively. We found that the expression levels of IL-6, *CCL5*, *GDF15*, *STC1*, *A2M*, *MT1X*, *MT1E*, *GPNMB*, and *VIP* were elevated in the experimental group ($p < 0.0001$, the specific expression of the gene is shown in **Supplementary Table 3**). This suggested that the RT-qPCR validation results were consistent with the RNA-seq results, and the sequencing results were credible (Fig. 3).

4. Discussion

EEF1A1 was successfully knocked down in U251 cells by siRNA transfection, and transcriptome sequencing was performed by RNA-seq high-throughput sequencing technology. Analysis of transcriptome sequencing data from experimental and control groups revealed that EEF1A1 knockdown caused 902 significantly differentially expressed genes. Analysis of GO and KEGG enrichment revealed that significantly up-regulated genes *IL-6*, *CCL5*, *GDF15*, *STC1*, *A2M*, *MT1X*, *MT1E*, *GPNMB*, and *VIP* were mainly enriched in the pathways which were associated with neuroinflammatory processes.

Our study showed that *GDF15* and *STC1* were significantly highly expressed in the cell-cell signaling path-

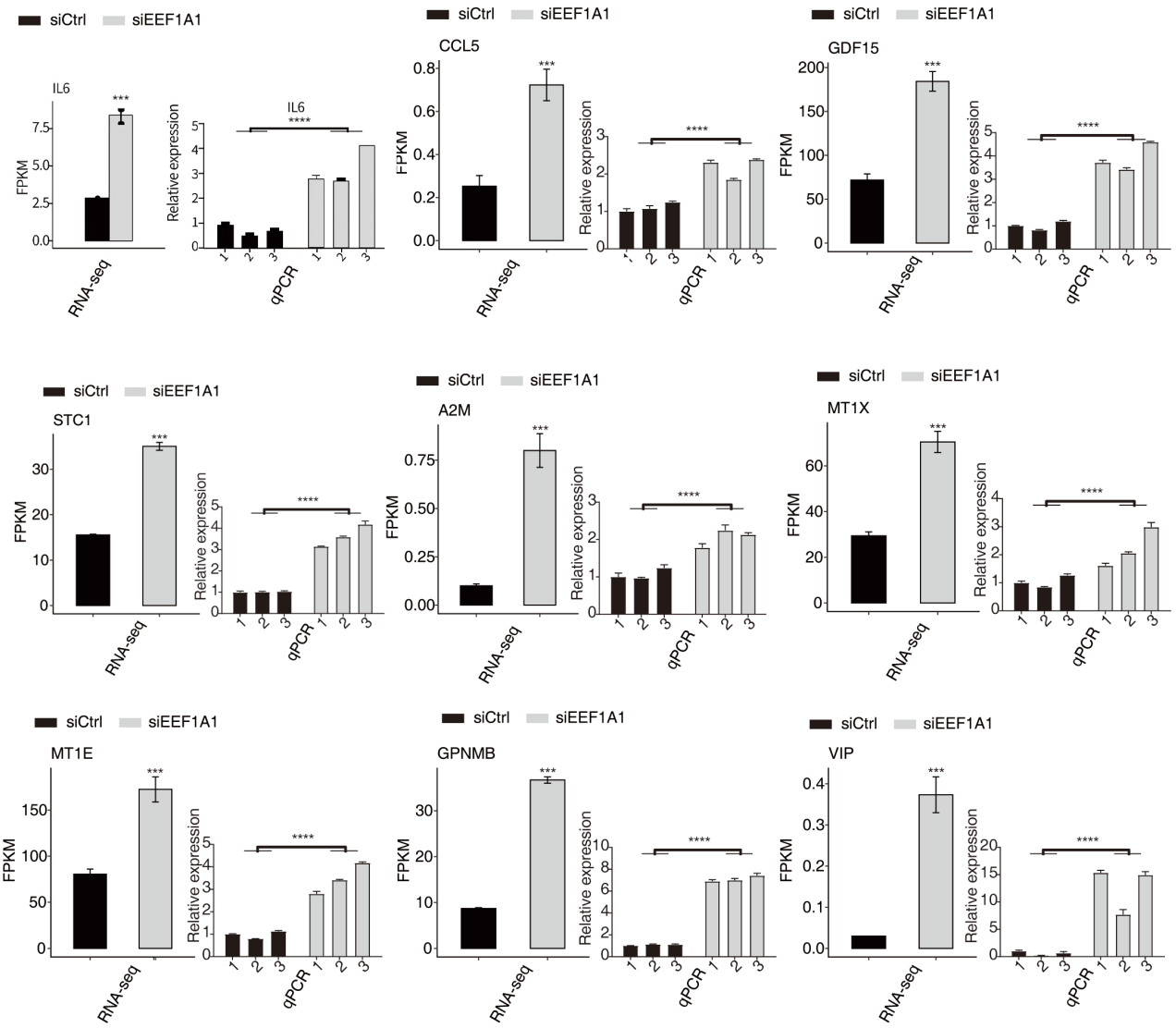

Fig. 3. The RT-qPCR validation results. Gene expression was quantified by RNA sequencing data and validated by RT-qPCR for these genes, respectively. The expression levels of IL-6, *CCL5*, *GDF15*, *STC1*, *A2M*, *MT1X*, *MT1E*, *GPNMB*, and *VIP* were elevated in the experimental group (Error lines represent mean $\pm$ standard error. ****$p < 0.0001$). RT-qPCR, Real Time - quantitative Polymerase Chain Reaction.

way. GDF15 belongs to the transforming growth factor - β (TGF-β) family, which is essential in the regulating of inflammation [27]. GDF15 inhibits the activation of the NF-κB pathway, and reduces the generation of inflammatory factors such as TNF-α [28]. It was found that the injection of GDF15 into the substantia nigra of experimental mice before the injection of 6-OHDA protected the mouse brain from 6-OHDA-induced damage, thereby preventing dopaminergic neuron depletion [27]. Machado *et al.* [29] studies have shown that endogenous GDF15 reduces the number of 6-OHDA-induced microglia in the nigrostriatal part of the experimental mouse. Maetzler *et al.* [30] found that GDF15 in cerebrospinal fluid was positively related to the age of PD onset, hoehn and yahr scale (H-Y) staging, and expression of the neurodegenerative marker Tau protein. Yao X *et al.* [31,32] showed that serum GDF15 was positively correlated with unified Parkinson's disease rating scale (UPDRS) scores in PD patients. The sensitivity of serum GDF15 for PD detection was 71.15%, and the specificity was 87.50%, which can be used as one of the PD biomarkers. STC1 is a glycoprotein hormone located in neuronal cells, macrophages, and cancer cells, etc. It was reported that STC1 has an essential function in neurological diseases by participating in inflammatory responses, mitochondrial oxidative phosphorylation, and calcium and phosphorus metabolism [33]. Sheikh-Hamad *et al.* [34] found that in macrophages, STC1 reduces superoxide by inducing uncoupling protein-2 (UCP2) generation. In en-

dothelial cells, STC1 inhibited the activation of inflammatory pathways such as JNK and NF-κB, reducing the superoxide generation. Shahim *et al*. [35] observed abnormal expression of STC1 in the cerebrospinal fluid of AD patients. Wang Pu *et al*. [33] found that in AD, STC1 overexpression attenuated oxidative stress-induced injury, and reduced neuroinflammation by inhibiting the ERK1/2 signaling pathway, indicating that STC1 may be an essential protective factor in AD. *A2M* is significantly overexpressed in the adverse regulatory signaling pathway of endopeptidase activity and the complement and coagulation cascade signaling pathways. A2M is prominent part of the innate immune system. Research has shown an increased plasma A2M level in AD patients [36]. Aberrant aggregation of α-synuclein is an important pathological feature of PD. α-synuclein induces activation of microglia, allowing increased production of ROS, cytokines and chemokines, leading to dopaminergic neuronal dysfunction [37]. Studies have reported that A2M can bind to α-synuclein binding and inhibit α-synuclein aggregation in PD. In addition, A2M is involved in regulating inflammatory processes [38].

In GO enrichment analysis, we found that EEF1A1 knockdown was followed by a considerable elevated the expression of gene *MT1X*, *MT1E* in the perinuclear region, which are involved in the inflammatory process, protects cells from injury caused by free radicals produced by oxidative stress in astrocytes. The expression of gene *VIP*, which has anti-inflammatory and antioxidant properties were also significantly increased in neurons. *GPNMB*, a gene with anti-inflammatory effects in macrophages and microglia, was also considerably increased. Studies have reported that metallothioneins (MTs) are considered neuroprotective "defense proteins". There are four main isoforms of MTs (1–4), among which MT1 is mainly located in astrocytes and can maintain intracellular homeostasis of zinc and copper ions, participate in inflammatory processes and protect cells [39].

A study showed that the protective effect of endogenous metallothionein (MT) against dopaminobenzone-induced neurotoxicity was observed after levodopa injection in a mouse model of PD with knockout MT1 [39]. Michael *et al*. [40] observed a considerable enhancement of *MT1E* and *MT1X* gene expression in nigrostriatal and frontal cortical astrocytes of PD patients, and acted as neuronal protectors. glycoprotein (transmembrane) nmb (GPNMB) is highly expressed in macrophages and microglia, and has anti-inflammatory effects [41]. GPNMB and CD44 (GPNMB's receptor) is increased in the substantia nigra of PD patients [42]. Budge *et al*. [43] found that *GPNMB* gene verexpression prevents dopaminergic neuronal degeneration in an MPTP-induced mouse model. GPNMB overexpression reduced the proliferation of glial cells after MPTP injection and prevented microglia morphological changes. Vasoactive intestinal peptide (VIP), as a neuropeptide neurotransmitter, is engaged in anti-inflammatory, neurophilic, and anti-apoptotic properties. Studies have reported that VIP inhibits microglia activation through inhibition of the COX-2/ PGE2 system, and inhibits microglia secretion of inflammatory chemokines such as TNF-α, IL-1β, IL-6, and NO, thereby protecting neuronal cells [44]. Olson *et al*. [45] found that VIP is participates regulating the inflammatory response, and protects against the deterioration of dopaminergic neurons. Broome *et al*. [46] found that VIP injection treatment inhibited the pro-inflammatory effects of rotenone in BV2 microglia and suppressed the increase of NO, CD11b, matrix metalloproteinase-9 (MMP-9, and IL-6, slowing the progression of PD.

In addition, *IL-6* and *CCL5* are significantly high expressed in the inflammatory response, TNF signaling pathway, and cytokine-cytokine receptor interaction signaling pathway. IL-6 is one of the significant inflammatory factors which is synthesized mainly in macrophages and glial cells. It involves in inflammatory processes, neurological function, and neurodegeneration [47]. CCL5 is expressed in endothelial and glial cells of the brain. It has participated in the recruitment of immune cells, and the regulation of the immune response [48]. The research indicates that CCL5 stimulates microglia activation, and increases NO secretion [49]. Elevated IL-6 levels were seen in the striatum, serum, and cerebrospinal fluid of PD patients [13]. Green *et al*. [50] found that IL-6 levels were positively associated with UPDRSIII scores, suggesting that higher IL-6 levels were related to more severe motor symptoms in PD patients. Tang Peng *et al*. [51] found that serum CCL5 levels in PD patients were positively correlated with H-Y staging, indicating that CCL5 may serve as a promising biomarker for PD severity evaluation.

5. Conclusions

In summary, this study demonstrated that EEF1A1 could regulate the expression of genes associated with PD neuroinflammation. Knockdown of EEF1A1 significantly up-regulated the genes *IL-6*, *GDF15*, *STC1*, *MT1E*, *GPNMB*, *CCL5*, *MT1X*, *A2M*, and *VIP*. Among them, *GDF15*, *STC1*, *MT1E*, *MT1X*, *GPNMB*, *VIP*, and *A2M* participated in delay PD neuroinflammation. At the same time, *IL-6* and *CCL5* were involved in exacerbating the PD neuroinflammation, implicating that EEF1A1 may participate in the regulation of the neuroinflammatory process of PD.

Abbreviations

EEF1A1, eukaryotic translation elongation factor 1 alpha 1; PARK2, Recombinant Parkinson Disease Protein 2; PD, Parkinson's disease; AD, Alzheimer's disease; RT-qPCR, Real Time - quantitative Polymerase Chain Reaction; 6-OHDA, 6-Hydroxydopamine hydrobromide; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; GAPDH, glyceraldehyde-3-Phosphate Dehydrogenase; DEGs, differential expressed genes; FPKM, expected fragments per kilobase of transcript per million fragments

mapped; FC, fold change; FDR, false discovery rate; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; IL-6, interleukin-6; GDF15, growth differentiation factor 15; STC1, stanniocalcin 1; MT1E, metallothionein 1E; GPNMB, glycoprotein (transmembrane) nmb; CCL5, chemokine (C-C motif) ligand 5; MT1X, metallothionein 1X; A2M, alpha-2-macroglobulin; VIP, vasoactive intestinal peptide.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

JM, JL and ZA designed the research study. ZA and JL performed the research. ZA, JL and YZ prepared the experiments and data analysis. ZA and JL wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.jin2205122.

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