MiRNA-103-3p targets Rab10 to activate the Wnt/β-Catenin signaling pathway to ameliorate corticosterone-induced injury in PC12 cells

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Research Article

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Abstract

As a key regulatory molecule in neurological disorders, the mechanism by which Rab10 exerts its protective effect in neuronal cells in depression is currently unknown. This research aimed to explore the function and mechanism of action of *Rab10*, a gene associated with neuroprotection, by using an *in vitro* model of depression. PC12 cells induced by corticosterone (CORT) were used to simulate depression *in vitro*. The viability of PC12 cells was detected using a CCK-8 assay, and the interaction between miRNA-103-3p and Rab10 was confirmed by bioinformatics combined with double luciferase and RNA Binding Protein Immunoprecipitation (RIP) experiments. The level of miRNA-103-3p and *Rab10* were detected using a quantitative PCR assay. The protein contents of Rab10, BDNF, CREB, P62, Beclin-1, Wnt3a, GSK3β, phosphorylated (p)-GSK3β, and β-catenin were detected by western blotting. The results indicated that the content of *Rab10* was downregulated in CUMS rats and CORT-induced PC12 cells. Bioinformatics combined with double luciferase and RIP experiments showed that miRNA-103-3p targeted *Rab10*. Overexpression of *Rab10* or silencing of miRNA-103-3p in CORT-induced PC12 cells activated the Wnt/β-catenin signaling pathway, upregulated the contents of BDNF, CREB, and Beclin-1, but downregulated the expression of P62 protein, whereas silencing *Rab10* based on silencing miRNA-103-3p reversed the effect of miRNA-103-3p. Overall, our data indicated that miRNA-103-3p targeted *Rab10* to activate the Wnt/β-catenin signaling pathway to increase cellular nerve plasticity and promote autophagy, thus resisting CORT-induced damage to PC12 cells.

1. Introduction

Depression is a common neurological and mental disease that is harmful to human physical and mental health. It is caused by the interactions of social, psychological, and biological factors[1]. The number of patients with severe depression has reached 246 million worldwide, and about 3.1% of them suffer from severe depression. Depression is highly prevalent, recurs easy easily, and is associated with high suicide rates. Moreover, it is challenging in terms of its recognition, consultation, and treatment. Its prevention and treatment have therefore become a serious public health problem[2, 3].

The specific pathogenesis of depression is not clear. Recent studies on the pathogenesis of this disorder suggest that neuron autophagy may be involved in its pathogenesis and treatment[4–8]. Studies have confirmed that antidepressants can have an antidepressant effect by regulating neuronal autophagy[9]. Autophagy is a process of engulfing and enveloping self-cytoplasmic proteins or organelles into vesicles and fusing with lysosomes to form autolysosomes, degrading the contents they envelop, thereby enabling the metabolic needs of the cell itself and the turnover of certain organelles. Autophagy also involves in neurogenesis and synaptic remodeling[10]. Because the occurrence of synaptic plasticity requires the reconstruction of synaptic protein composition, the degradation of synaptic proteins is involved in this crucial process[11]. In recent years, we found that autophagy targeted neuronal proteins, and the imbalance of autophagy led to the accumulation of misfolded proteins and damaged organelles, which led to an imbalance of neuronal plasticity and further led to neuronal dysfunction and even death.

Therefore, it has been suggested that autophagy and neuroplasticity possibly take part in the happening and progress of depression[12–14].

The happening and progress of depression are usually the results of long-term interactions between genetic and environmental factors, with a complex molecular regulation mechanism[15]. The regulatory patterns of complex networks are often formed between genes and protein molecules through extensive and nonlinear interactions. Using traditional single gene or single protein “fragmentation” studies, it has therefore been difficult to identify the pathogenesis of depression and the effects of antidepressants, while global and quantitative full transcriptome studies can identify the sum of all RNAs that can be transcribed by specific cells, tissues, or organs at a whole tissue level under physiological or pathological conditions[16]. These methods can identify the functional elements of the genomes of cells, tissues, or organs, as well as reveal their molecular components, and can also they can predict the biological processes and the mechanisms of diseases. Several studies have therefore characterized the pathogenesis of diseases and the mechanisms of drug actions from the perspective of the transcriptome[17].

The hippocampus act a vital role in emotion, memory, learning, endocrine, and visceral activities, and is part of the limbic system of the brain[18]. Because the hippocampus contains high levels of glucocorticoid receptors and glutamate, it is an active role in the regulation of the Hypothalamic Pituitary Axis (HPA) effect, which makes it more vulnerable to external pressures. Studies have found that chronic and severe stress have been shown to impair dominant memory in animal models of hippocampal-dependent depression [19]. Stress can also reduce the branching and plasticity of the neuronal dendritic hippocampus. The biological mechanism of depression is therefore usually studied from the perspective of hippocampal tissue[20].

Rab10 is a member of the small GTP enzymes of the Rab family, which is a key regulator of vesicle transport[21]. Rab10 is widely expressed in various neuronal cells in the brain. Rab protein is cell cycle-controlled and requires GDP-GTP exchange promoted by the Rab guanine nucleotide exchange factor. Nevertheless, Rab10 plays a relatively nonspecific role in intracellular transport, which is different from most other Rab proteins; it performs a diverse function and has a variety of subcellular localizations[22, 23]. Rab10 take a crucial part in occurrence; when both alleles are deleted, the mouse is embryonically dead. The function of Rab10 is also related to the morphology and polarization of neurons, and take an essential part in axonal occurrence and dendritic branch formation. During neuronal development, Rab10 binds to the plasma membrane precursor vesicles, which are connected to Kinesin1 through c-jun N-terminal protein kinase interacting with protein 1. This complex jointly mediate the anterograde transport of Rab10-positive vesicles to the end of the axon and promotes axonal growth. Therefore, Rab10 act a significant role in maintaining the proper functioning of neurons[24].

MicroRNA (miRNA) is a short, 17 – 23 nucleotide non-coding RNA that can complement and bind to the 3'-UTR of the target gene mRNA in vivo, to inhibit the expression of the target gene[25, 26]. Because of their short sequences and specificity for their target genes, miRNA is promising to become a new target for the

therapy of central nervous system (CNS) diseases[27, 28]. Chromatin Immunoprecipitation (Chip) assay found that miR-103-3p was highly expressed in the central nervous system and further upregulated after depression, suggesting that it take an essential part in the regulation of gene expression in the central nervous system[29]. Other researchers found that increasing the level of miR-103-3p has a certain inhibitory function on the proliferation of nerve cells, induced apoptosis, and inhibited differentiation, suggesting that the regulation of miR-103-3p may be a new treatment for neurological diseases[30].

In the early stages, transcriptome sequencing found that there was differential expression of Rab10, which is related to neural plasticity and autophagy, but the mechanism by which Rab10 protects nerve cells in depression is unclear. Therefore, this research aimed to explore the specific mechanism by which miRNA-103-3p regulation of Rab10 exerts a protective effect on neuronal cells in an in vitro model of corticosterone (CORT)-induced PC12 cells simulated depression.

2. Materials And Methods

2.1 Analysis of differentially expressed genes

Analysis of differentially expressed genes between samples was performed by the edgeR function, whereby P values were determined and corrected. Meanwhile, according to the fragments per kilobase of the exon model per million mapped read values to calculate the differential expression multiple (Fold change). Comparing samples of the Control and CUMS groups, the screening conditions: (1) P-value $\leq$ 0.05; (2) Fold change $\geq$ 2.

2.2 Gene Ontology (GO) enrichment analysis

GO sets up a set of control words with dynamic form. There are three categories in the GO database, namely, Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). Map the GO information of the protein (Uniprot database) according to the identified protein ID, and the functional classification of the protein was carried out. For the GO nodes, all the corresponding protein numbers are listed, and the secondary classification of expressed proteins is plotted respectively.

2.3 Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

The KEGG statistics of distinguishingly expressed genes were used to determine the number of distinguishingly expressed genes in each pathway. The significantly distinguishingly expressed genes were selected, and then a statistical algorithm such as hypergeometric distribution was used to calculate the p-value of whether the pathway was significant based on the number of genes that differed from that pathway (also Q-value was obtained in general), which was ranked by the magnitude of Q-value.

2.4 PC12 cell culture

The PC12 cell line was derived from rat pheochromocytoma (Cybertron Biotechnology, Shanghai, China). PC12 cells in 100 μg/mL streptomycin, 100 U/mL penicillin, and 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) were cultured in DMEM medium (Hyclone Laboratories, South Logan, UT, USA) and cultured in an incubator (5% CO_2, 37°C). Similarly, for the preparation of CORT solution, a certain amount of corticosterone powder (TCI, Tokyo, Japan) was weighed, mixed under sterile conditions with a certain volume of dimethyl sulfoxide solution (Gibco, Carlsbad, CA, USA), and configured into a mother liquor of 1,000 μM. The mother liquor was diluted with medium to the desired working concentrations (100, 200, 300, 400, and 500 μM) before use. It was then packed into a single dosage and stored at -80°C.

2.5 The siRNA and plasmid transfections

Plasmid and siRNA were introduced into cells by liposome transfection. Before transfection, the cells were digested, centrifuged, counted, and then evenly inoculated into a six-well culture plate. The next day, transfection was conducted when the cell density grew to a specified level (more than half and less than 80% confluency). Lipofectamine™ 3000 (Thermo Fisher Scientific, Waltham, MA, USA) was used to transfect the cells for 48 ~ 72 hours. Before transfection, the old complete culture medium was thoroughly removed, and phosphate-buffered saline (PBS; Gibco, Carlsbad, CA, USA) was used to wash the plates. The transfection-related reagents were prepared and marked as A and B tubes. For A tubes, each well was 5 μL Lipo3000 transfection reagent plus 125 μL DMEM medium mixed. For B tubes, in each well, 125 μL DMEM medium, 1 – 2.5 μg DNA, and 2 – 5 μL P3000 (transfection of siRNA was without P3000) were mixed. The liquid in the A tube was mixed with the liquid in the B tube and slowly added to wells in 6-hole plates. After addition, the contents were mixed by shaking the plates back and forth slightly, followed by incubation at a constant temperature for 48 ~ 72 hours. Q-PCR and western blotting were used to assess interference efficiencies.

2.6 Experimental protocol design

CORT-induced PC12 cells were individually infected with Lipofectamine™ 3000 transfection reagent and oe-NC, sh-NC, oe-Rab10, sh-Rab10, inhibitor-NC, or miRNA-103-3p inhibitor, separate or in combination, for 48 hours. All plasmids and small interfering fragments were purchased from Guangzhou Ruibo Biotechnology (Guangzhou, China).

2.7 Cell viability assay and observation of cell morphology

The effects of CORT on cell viability were detected using the CCK8 (Beyotime Institute of Biotechnology, Suzhou, China) method. The cells (1.8×10^3/well) were inoculated into 96-well plates (three plates). After 24 hours of culture, the degree of cell confluency was about 50%, then 10 μL of CCK8 and 90 μL DMEM complete medium were added. After being cultured for 2 ~ 4 hours in an incubator, the absorbance of the cells was tested using an enzyme labeling detection instrument at 450 nm (BioTek, Winooski, VT, USA).

2.8 Dual luciferase assay

To construct Rab10-WT and Rab10-MUT luciferase reporters, the 3'-UTRs of Rab10-WT and Rab10-MUT were amplified and inserted into the pmirGLO vector (Invitrogen, Carlsbad, CA, USA). Rab10-WT or Rab10-

MUT and miR-103-3p, miR-NC, anti-NC, or anti-miR-103-3p were co-transfected into chondrocytes using Lipofectamine™ 3000 (Thermo Fisher Scientific). At 48 hours after transfection, dual-luciferase reporter gene assays were performed to measure the luciferase activity using the Dual-Luciferase Reporter Assay System kit (Promega, Madison, WI, USA).

2.9 Anti-Ago2 RIP (RNA Binding Protein Immunoprecipitation) assay

RIP Kit (Millipore, Bedford, MA, USA) was used to identify the interaction of miRNA-103-3p with Rab10. PC12 cells were transfected with miR-NC or miR-103-3p for 48 ~ 72 hours. The cells were then incubated with a negative control antibody (IgG) or anti-Ago2 antibody, and the relative enrichment of Rab10 was measured by Q-PCR.

2.10 Q-PCR

Extraction of RNA from tissues involved grinding the tissues in a grinding disruptor, then approximately 1 mL TRIzol lysis solution (Tiangen Biotech, Beijing, China) was added for every 50 mg of the tissue sample. To extract the RNA from cells, added 1 mL TRIzol lysis solution to each well. A total of 800 μL of 75% ethanol was added, then cells were centrifuged and the supernatant was discarded. A total of 20 μL diethylpyrocarbonate (DEPC) water was then added, followed by mixing with gentle pipetting. The extracted RNA of each group was later reverse-transcribed into complementary DNA (cDNA). Each group of cDNAs and amplified primers (Ribobio, Guangzhou, China) were co-incubated with SYBR fluorescent dye (Tiangen Biotech). The mixed solution was later added to an 8-tube strip and put in a fluorescence quantitative PCR instrument for PCR amplification. U6 was used as an internal reference for miRNA-103-3p, GAPDH was used as an internal reference for Rab10, and the relative abundances of RNAs were computed using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are shown in Table 1.

Table 1
Primer sequences for Q-PCR

Gene	Primer sequence (5′-3′)
Rab10	F: GCTGAAGACATCCTCCGAAAGACC
	R: CCGTCACGCCTCCTCCACTG
miRNA-103-3p	F: GAGCAGCATTGTACAG
	R: GTGCAGGGTCCGAGGT
GAPDH	F: ACAACTTTGGTATCGTGGAAGG
	R: GCCATCACGCCACAGTTTC
U6	F: GCTTCGGCAGCACATATACTAAAAT
	R: CGCTTCACGAATTTGCGTGTCAT

2.11 Western blotting

The protein samples were boiled at 100°C and electrophoresed using 10%–12% SDS-PAGE, and then placed on polyvinylidene fluoride films (Bio-Rad, Hercules, CA, USA), then incubated with blocking solution. Subsequently, the membrane was combined with antibodies to: Rab10, BDNF, CREB, P62, Beclin-1, Wnt3a, GSK3β, phosphorylated (p)-GSK3β, β-catenin, and β-actin (Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. Subsequently, we rinsed the membrane with TBST buffer (three times every 15 minutes), then incubated it with goat anti-rabbit antibody (Abcam, Cambridge, MA, USA) at 26 ± 2°C. Next, the film was washed with TBST (three times every 15 min). Finally, the western blot bands were visualized using electrochemiluminescence reagents (Bio-Rad).

2.12 Statistical analysis

All data were analyzed using SPSS statistical software for Windows, version 18.0 (SPSS, Chicago, IL, USA). The results showed a normal distribution with homogenous variance, expressed as the mean ± standard deviation. We used a single sampling *t*-test to compare data from the two populations and a one-way analysis of variance. $P < 0.05$ was considered a statistically significant difference.

3. Results

3.1 The transcriptome of CUMS rats

We identified the molecular characteristics of depression in CUMS rats at the gene transcriptional level by comparing full transcriptome sequencing and data analysis of the hippocampal tissues of the control (n = 4) and CUMS groups (n = 4). The results showed that the hippocampus of the Control and CUMS groups present a great deal of differentially expressed genes (Fig. 1A and B). Significant differentially expressed genes in different groups were analyzed by GO annotation and GO term enrichment analysis. The consequence indicated that the GO term was mainly involved in BPs, involving positive regulation of the cell cycle, positive regulation of neuron differentiation, etc. (Fig. 1C). In the control and CUMS groups, the differentially expressed genes were enriched and a significantly enriched KEGG pathway was found. Among them, the pathway with many differentially expressed genes involved the Wnt signaling, MAPK signaling, and Hippo signaling pathways, etc. (Fig. 1D). RNA-seq results show that the expression of Rab10 in the hippocampus of Control rats was significantly higher than that of CUMS rats ($P < 0.05$) (Fig. 1E). Q-PCR and Western blot were used to determine the expression levels of Rab10 in Control versus CUMS groups the results were consistent with full transcriptome sequencing ($P < 0.05$) (Fig. 1F-H).

3.2 Construction of a depression model in vitro and verification of Rab10 interference efficiency

To identify the function of Rab10 in depression, CORT was used to stimulate PC12 cells to establish an *in vitro* model of depression. The outcome of the CCK-8 experiment declared that different concentrations of

CORT reduced the viability of cells to different degrees after incubation for 24 hours ($P < 0.05$). When PC12 cells were cultured with 300 μM CORT for 24 hours, the viability of PC12 cells decreased to 50% ($P < 0.05$). In the follow-up experiment, 300 μM CORT was used (Fig. 2A). Similarly, in PC12 cells, Q-PCR results showed that CORT stimulation at 300 μM decreased the mRNA expression of Rab10 ($P < 0.05$) (Fig. 2B). Western blot assay indicates that 300 μM CORT stimulation decreased the expression of Rab10 protein compared with Control cells ($P < 0.05$) (Fig. 2C and D). To identify the biological function of Rab10, we transfected the Rab10 overexpression plasmid into PC12 cells stimulated by CORT. Compare with the CORT + oe-NC group, the western blotting assay indicated that the expression of Rab10 protein in the CORT + oe-Rab10 group was higher ($P < 0.05$) (Fig. 2E and F).

3.3 Effects of overexpressing Rab10 on the expressions of neuroplasticity and autophagy-related proteins in CORT-induced PC12 cells

The transcriptome data and literature analysis showed that Rab10 was a key regulator of vesicle transport, and the function of Rab10 was also related to the morphology and differentiation of neurons. The expressions of neuroplastic-related proteins in PC12 cells were detected by western blotting, which showed that CORT stimulation significantly downregulated the expressions of BDNF and CREB proteins in PC12 cells ($P < 0.05$), while overexpression of Rab10 significantly increased the expressions of BDNF and CREB proteins in cells. Compared with the CORT + oe-NC group, the contents of BDNF and CREB proteins in the CORT + oe-Rab10 group significantly increased ($P < 0.05$), with the expressions of autophagy-related proteins detected by western blotting. Furthermore, the results showed that CORT stimulation significantly increased the expression of P62 protein and downregulated the expression of Beclin-1 protein ($P < 0.05$). Overexpression of Rab10 downregulated the expression of P62 protein and upregulated the expression of Beclin-1 protein. Compared with the CORT + oe-NC group, the level of P62 protein in the CORT + oe-Rab10 group decreased, while the level of Beclin-1 protein increased ($P < 0.05$) (Fig. 3).

3.4 Effects of overexpressing Rab10 on the Wnt/β-catenin signaling pathway in cells

The assay of the transcriptome, mass spectrometry, and later verification showed that the Wnt/β-catenin signal pathway was regulated by Rab10 during the depression. Results indicated that CORT stimulation significantly downregulated Wnt3a, p-GSK3β, and β-catenin protein expressions in PC12 cells by western blotting ($P < 0.05$). The protein expressions of Wnt3a, p-GSK3β, and β-catenin were upregulated in the CORT + oe-Rab10 group compared with the CORT + oe-NC group ($P < 0.05$) (Fig. 4).

3.5 Interaction of miRNA-103-3p when targeting Rab10

We also conducted bioinformatics analysis to explore the upstream miRNA targeting Rab10, which showed that Rab10 may be a potential target for miRNA-103-3p (Fig. 5A). Compared with rats in the control group, Q-PCR results indicated that miRNA-103-3p was significantly upregulated in the

hippocampus of rats in the CUMS group ($P < 0.05$) (Fig. 5B). A double luciferase report and anti-Ago2 RIP assay showed that Rab10 was the direct target of miRNA-103-3p ($P < 0.05$) (Fig. 5C-E). These results revealed that miRNA-103-3p targeting Rab10 affected the CORT-induced PC12 cells.

3.6 The miRNA-103-3p regulates Rab10 expression.

Q-PCR results showed that CORT stimulation significantly upregulated miRNA-103-3p expression in PC12 cells ($P < 0.05$) (Fig. 6A). A miRNA-103-3p inhibitor was transfected into CORT-induced PC12 cells. Compared with the CORT + inhibitor NC group, Q-PCR showed that miRNA-103-3p expression was downregulated in the CORT + miRNA-103-3p inhibitor group ($P < 0.05$) (Fig. 6B). Similarly, the western blotting assay expressed that CORT stimulation significantly downregulated the expression of Rab10 protein in cells ($P < 0.05$). However, the expression of Rab10 protein was upregulated after transfection of the miRNA-103-3p inhibitor, that is, the expression of Rab10 in the CORT + miRNA-103-3p inhibitor group was significantly upregulated compared with the CORT + inhibitor-NC group ($P < 0.05$) (Fig. 6C and D). Similarly, the western blot assay revealed that the protein expression of Rab10 was significantly downregulated in the CORT + sh-Rab10 group when compared with the CORT + sh-NC group ($P < 0.05$) (Fig. 6E and F).

3.7 Effects of Rab10 regulation by miRNA-103-3p on CORT-induced expression of neuroplasticity and autophagy-related proteins in PC12 cells

Detection of the expressions of neuroplastic-related proteins in cells using western blotting. The results declared that protein expressions of BDNF and CREB were increased in the CORT + miRNA-103-3p inhibitor group when compared with the CORT + inhibitor-NC group ($P < 0.05$). Compared with the CORT + miRNA-103-3p inhibitor + sh-NC group, the protein expressions of BDNF and CREB were significantly downregulated in the CORT + miRNA-103-3p inhibitor + sh-Rab10 group ($P < 0.05$). When detecting autophagy-related protein expression in cells using western blotting, the results declared that compared with the CORT + inhibitor-NC group, P62 protein was decreased, whereas Beclin-1 protein was increased in the CORT + miRNA-103-3p inhibitor group ($P < 0.05$). Compared with the CORT + miRNA-103-3p inhibitor + sh-NC group, the level of P62 protein in the CORT + miRNA-103-3p inhibitor + sh-Rab10 group increased, while the content of Beclin-1 protein decreased ($P < 0.05$) (Fig. 7).

3.8 Effect of miRNA-103-3p regulation of Rab10 on the Wnt/β-catenin signaling pathway in CORT-induced PC12 cells

Detection of the expressions of related proteins in the Wnt/β-catenin signaling pathway by western blotting showed that the protein levels of Wnt3a and p-GSK3β and β-catenin in the CORT + miRNA-103-3p inhibitor group were significantly higher than those in the CORT + inhibitor-NC group ($P < 0.05$). Compared with the CORT + miRNA-103-3p inhibitor + sh-NC group, the levels of Wnt3a, p-GSK3β, and β-catenin

protein in the CORT + miRNA-103-3p inhibitor + sh-Rab10 group were significantly decreased ($P < 0.05$) (Fig. 8).

4. Discussion

This experiment indicated that compared with the Control group, the mRNA expression and protein content of Rab10 in the CUMS group were downregulated. Overexpression of Rab10 in CORT-induced PC12 cells significantly improved the level of neuroplasticity and upregulated the level of autophagy, to improve cell injury. Bioinformatics combined with double luciferase and RIP experiments showed that miRNA-103-3p targeted Rab10, and miRNA-103-3p regulated Rab10 to activate the Wnt/ β-catenin signaling pathway to resist CORT-induced damage in PC12 cells.

With an in-depth discussion of the pathophysiological mechanism of depression, increasing studies have reported the gene expression characteristics of depression at the genome-wide level, which aided in the further discovery of the pathophysiological mechanism of depression. A genome-wide association study found that bipolar disorder caused changes in genes such as *CSMD1, SYNEI, ADCY2, NCALD, WDR60, SCN7A*, and *SPAG16*, which are involved in the calcium signal transduction pathway, neuropathic pain signal transduction, neuronal CREB signal transduction, glutamate receptor signal transduction, and axonal guidance signal transduction[31]. In this research, it was declared that there was a great deal of differentially expressed genes in the hippocampus of normal and CUMS rats when using full transcriptional sequencing, and the enrichment of differentially expressed genes showed that the GO term was greatly taken part in BPs, involving positive regulation of the cell cycle, positive regulation of neuron differentiation, etc. The KEGG pathway with significant enrichment of differentially expressed genes involved the Wnt signaling, MAPK signaling, and Hippo signaling pathways. When combined with full transcriptional group sequencing and Q-PCR verification, it was found that expression of the *Rab10* gene in the hippocampus of the Control group was higher than that of the CUMS group, showing that Rab10 acts a vital regulatory role in depression.

Highly differentiated PC12 cells have the characteristics of typical neuroendocrine cells in morphology and function, so they are widely used in the study of depression[32]. For example, a model of PC12 cells damaged by a high concentration of CORT has been widely used. Different types of antidepressants have protective effects on CORT-damaged PC12 cells[33, 34]. In the present study, the CCK-8 assay was used to show that CORT affected the activity of PC12 cells, that is, after 300 μM CORT stimulation of PC12 cells for 24 hours, cell survival decreased by 50%. Similarly, the stimulation with 300 μM CORT significantly downregulated the expression of Rab10 protein in PC12 cells (consistent with the results of hippocampal tissue verification). It is suggested that the CORT-induced PC12 cell model can be used in the study of depression *in vitro*. Studies have found that decreased Rab10 expression leads to a decreased ratio of Aβ 42/Aβ 40 in the plasma of patients with Alzheimer's disease, which aggravates the condition, and showed that Rab10 is a potential therapeutic target for neurological diseases[35].

In this study, bioinformatics combined with double luciferase and RIP experiments showed that miRNA-103-3p targeted Rab10, and the expression of miRNA-103-3p was upregulated in the hippocampus of CUMS rats and PC12 cells induced by CORT, suggesting that miRNA-103-3p may involve in depression by regulating the expression of Rab10. MiR-103-3p highly overlaps with nearly 20% of the mRNA in the central nervous system, most of which are membrane-related and metabolism-related genes. Some studies have shown that inhibition of miR-103-3p reduced brain injury after ischemic stroke and improved neurological impairment after ischemic stroke[29, 30].

Neuroplasticity refers to adaptive changes related to the function of the brain with a variety of internal and external stimuli, involving numerous changes in the structure and function of neurons. The mechanism of neural plasticity includes synaptic morphological changes and increased gene expression[36, 37]. The results showed that CORT stimulation significantly downregulated the expressions of BDNF and CREB in PC12 cells, while overexpressed Rab10 significantly upregulated the contents of BDNF and CREB in PC12 cells. Knockdown of miRNA-103-3p with transfected miRNA-103-3p inhibitor also significantly elevated BDNF and CREB protein expressions in cells, but silencing Rab10 significantly reversed the effects of miRNA-103-3p on neuroplasticity-related proteins in cells. The neurotrophin hypothesis suggests that BDNF acts as a biomarker for depression and that low levels, of low-functioning BDNF, are highly susceptible to the development of depression[38]. Also acting as an important neurotrophic factor in the CNS, regulating the expression of BDNF exerts an irreplaceable effect on the growth and survival of neurons. Studies have reported extremely low levels of BDNF in the hippocampus of depressed patients who presented suicidal behavior; Whereas BDNF content in the hippocampus was upregulated in depressed patients after regular antidepressant treatment compared with those who did not[39]. The results suggested that miRNA-103-3p improved the neuroplastic function of PC12 cells by regulating the level of Rab10 and increasing the content of BDNF and CREB.

Autophagy was acted an essential role in the pathogenesis and treatment of depression[40]. For example, autophagy selectively targets damaged organelles and toxic proteins, such as mitochondrial autophagy, so the selective removal of damaged mitochondria by autophagy inhibits apoptosis[41]. It was found that CORT stimulation significantly decreased the autophagy progress of PC12 cells, and upregulated of Rab10 or silencing of miRNA-103-3p significantly downregulated the level of P62 protein and upregulated the level of Beclin-1 protein, thus promoting autophagy. However, silencing Rab10 after transfection with miRNA-103-3p inhibitor to inhibit miRNA-103-3p attenuated the function of miRNA-103-3p on the level of autophagy. Consistent with this result, some studies have reported the therapeutic effects of some antidepressants, which upregulate the level of autophagy; tricyclic antidepressant amitriptyline and selective 5-HT reuptake inhibitor citalopram can increase the level of autophagy of astrocytes and neurons and inhibit neuronal apoptosis in depressed rats[42].

The Wnt signal transduction pathway controls various biological phenomena in the development and adulthood of all animals[43]. Wnt signaling plays an important role in neuronal synaptic formation and remodeling, dendritic growth and branch formation, nerve transmission, neuroplasticity, neurogenesis, and neuroprotection[44].

Analyzing study data we draw that upregulated expression of Rab10 or silencing of miRNA-103-3p significantly upregulated the content of Wnt3a, p-GSK3β, and β-catenin proteins in PC12 cells, thus activating the Wnt/β-catenin signaling pathway to decrease the damage of PC12 cells induced by CORT. Silencing Rab10 based on the transfection of miRNA-103-3p inhibitor to inhibit miRNA-103-3p significantly reversed the effect of miRNA-103-3p on the Wnt/β-catenin signaling pathway. GSK3β is a serine/threonine protein kinase. Without a Wnt signal, GSK3β can phosphorylate β-catenin (adding a phosphate group to the N-terminal serine/threonine residue), then covalently modified by β-TRCP protein and degraded by the proteasome. Free β-Catenin can enter the nucleus to regulate gene expression, β-Catenin interacts with E-Cadherin at the cell junction, and its abnormal expression or activation may cause disease[45, 46].

5. Conclusions

Overall, data from this study showed that miRNA-103-3p targeted the Rab10-activated Wnt/β-catenin signaling pathway to decrease the damage of PC12 cells induced by CORT. In addition, overexpression of Rab10 or silencing of miRNA-103-3p significantly improved the neuroplasticity of PC12 cells induced by CORT and promoted the level of autophagy; while silencing Rab10 based on silencing miRNA-103-3p reversed the effect of miRNA-103-3p. Our results, therefore, provide insights into the potential therapeutic value of miRNA-103-3p targeting Rab10 and its neuroprotective role in depression; however, its exact mechanism of antidepressant-like effect needs to be determined.

Declarations

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Author contributions Xin Zhang, Ye-Ming Zhang, and Yuan-Xiang Zhang designed the project and wrote the manuscript. Ye-Ming Zhang, Yuan-Xiang Zhang, and Rong Fan established the cell model. Ye-Ming Zhang and Yuan-Xiang Zhang performed the Western blot and Q-PCR experiments. Ye-Chao Lv, Xiao-Xu Liu, Rong Fan and Xin Zhang assisted in the analyses of data.

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Data availability All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval These experiments in our research were countenanced by the Ethics Committee of Wannan medical college.

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Figures

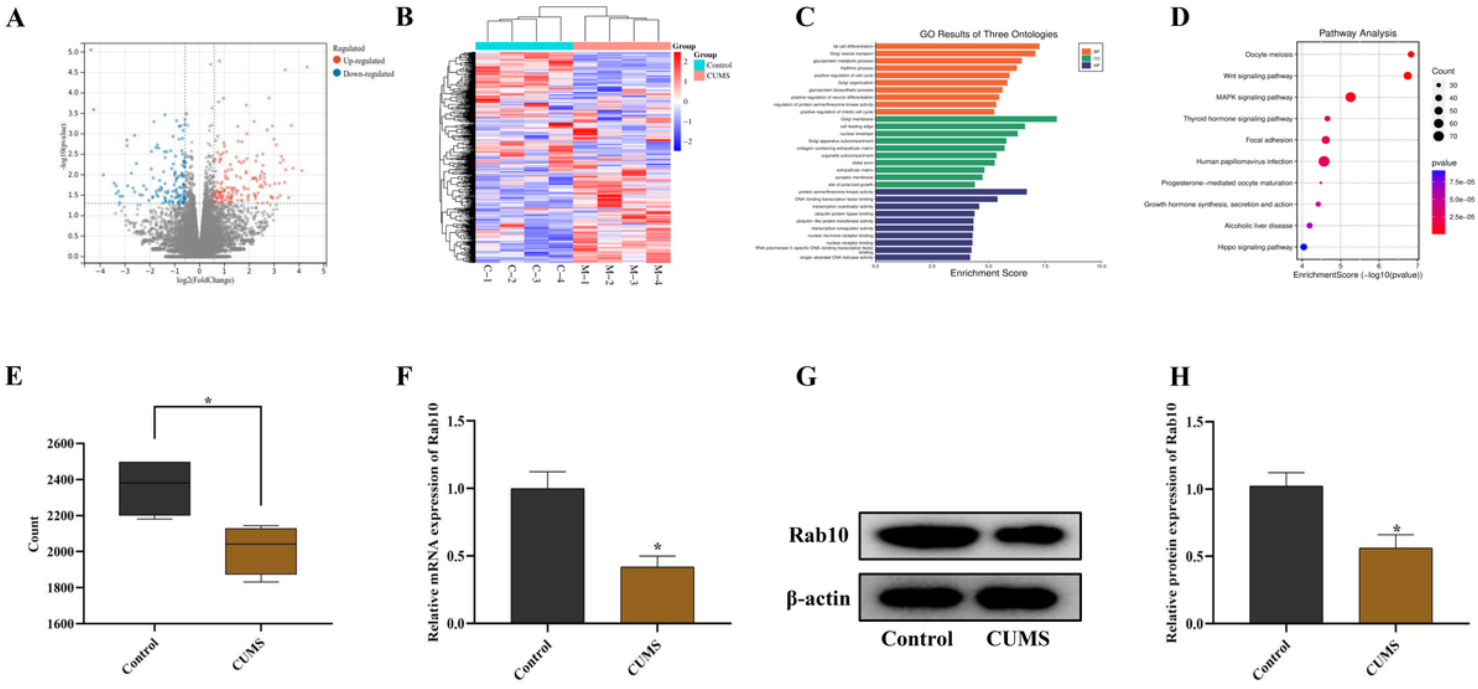

Figure 1

Full transcriptome sequencing found that *Rab10* was a key regulatory gene in depression. A Differential gene volcano map. B Differential gene heat map. C Go enrichment analysis. D KEGG enrichment analysis. E Rab10 expression in the RNA-seq. F Q-PCR detection of Rab10 expression in the rat hippocampus. G

and H Western blot analysis normalized immunoblots and quantitation of Rab10 protein expression normalized to β-Actin in the rat hippocampus. $^{*}P < 0.05$ vs. the Control.

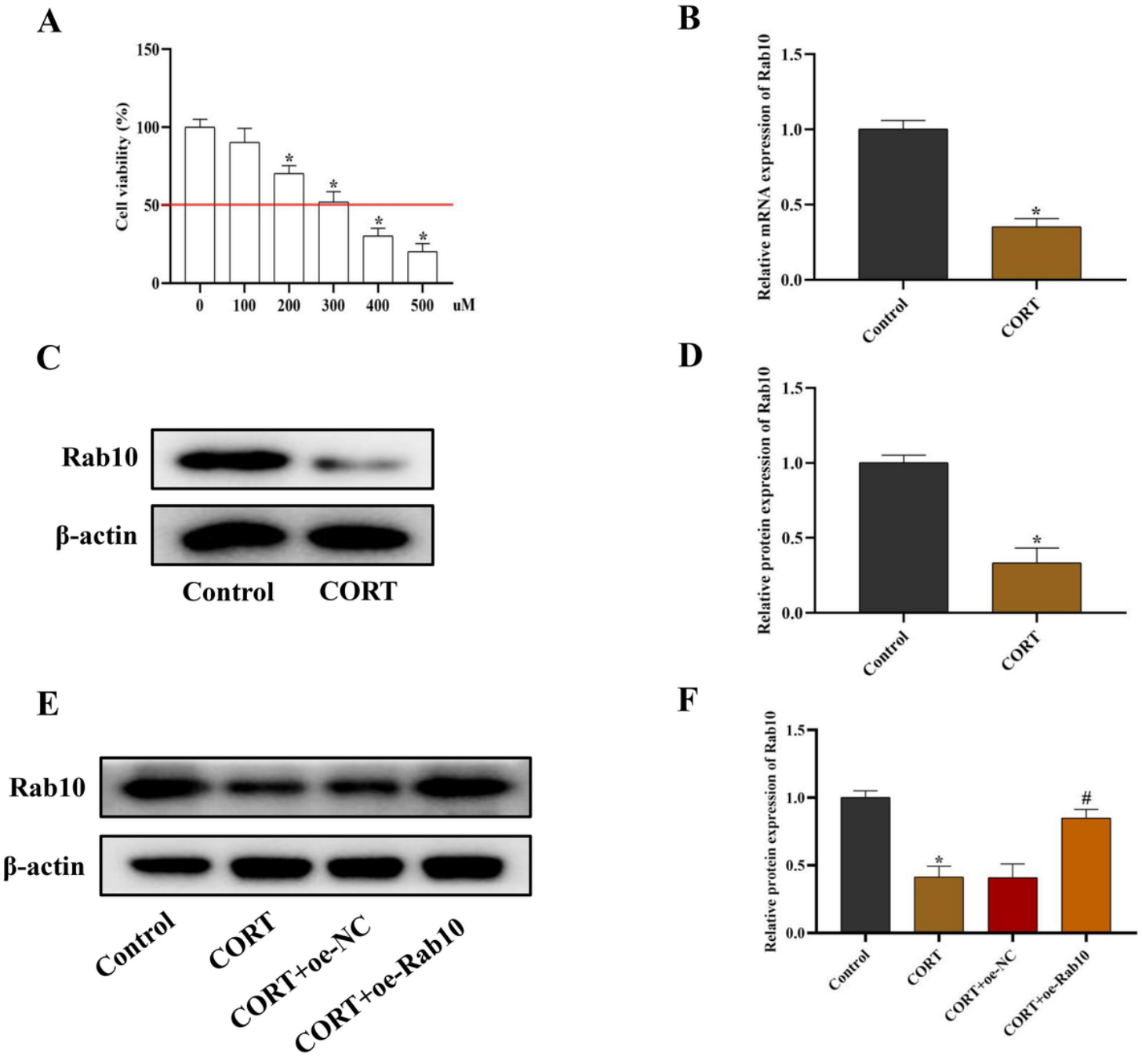

Figure 2

Rab10 expression was downregulated in the CORT-induced PC12 cell apoptosis model. A The CCK-8 experiment was conducted to determine the optimum concentration of CORT. B Detect the expression of Rab10 in PC12 cells after CORT stimulation by Q-PCR. C Immunoblots of Rab10 and β-actin in Control and CORT groups by western blot. D Rab10 statistical chart in Control and CORT groups. E Immunoblots of Rab10 and β-actin in Control, CORT, CORT+oe-NC, and CORT+oe-Rab10 groups by western blot. F

Rab10 statistical chart in Control, CORT, CORT+oe-NC, and CORT+oe-Rab10 groups. $^{*}P < 0.05$ vs. the control. $^{\#}P < 0.05$ vs. CORT+oe-NC.

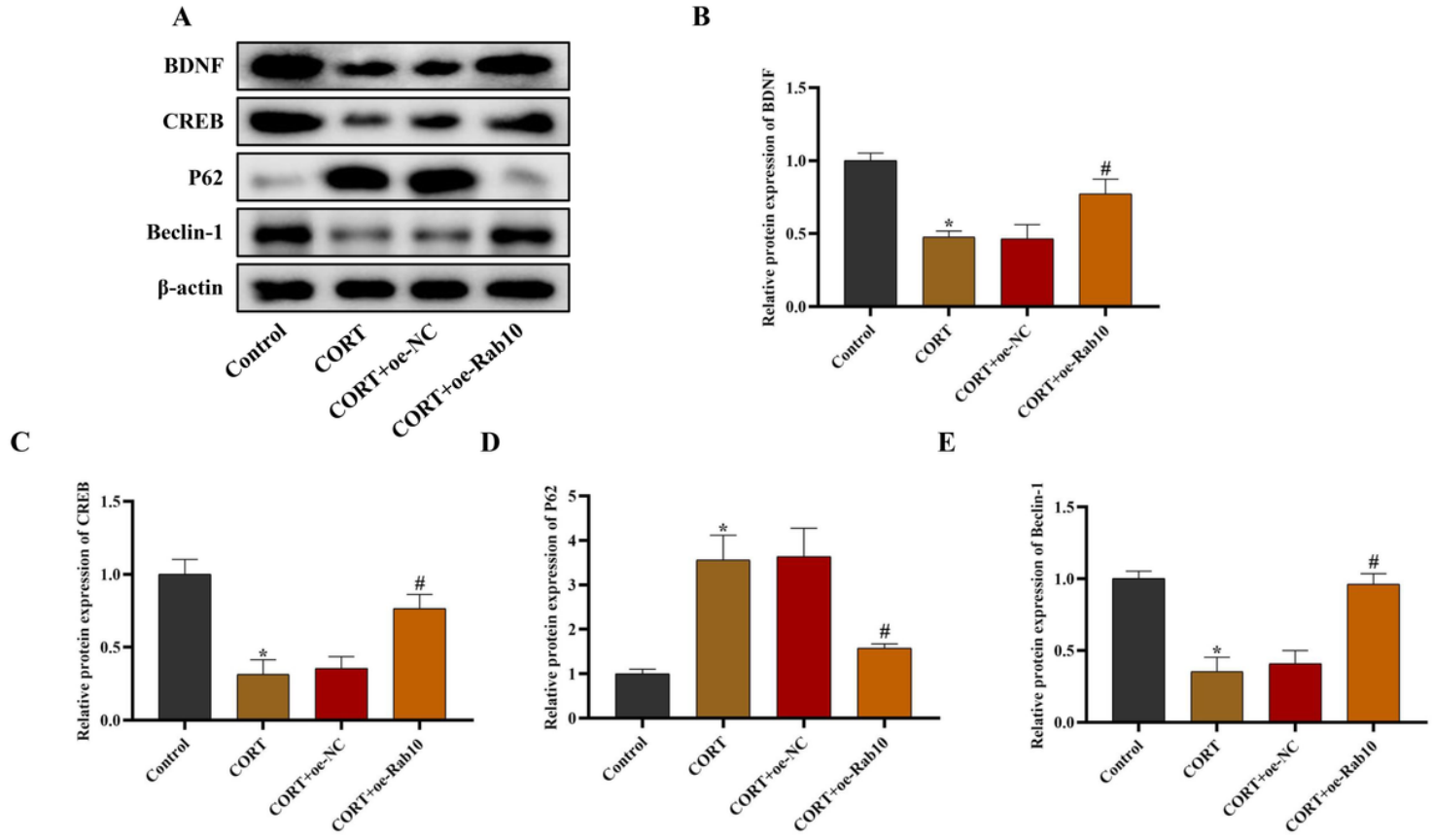

Figure 3

Western blot detection of the expressions of neural plasticity and autophagy-related proteins in cells. A-E Immunoblots and quantitation of BDNF, CREB, P62, and Beclin-1 protein expressions normalized to β-actin in PC12 cells after treatment with oe-Rab10, when measured using western blot analyses. $^{*}P < 0.05$ vs. the control. $^{\#}P < 0.05$ vs. CORT+oe-NC.

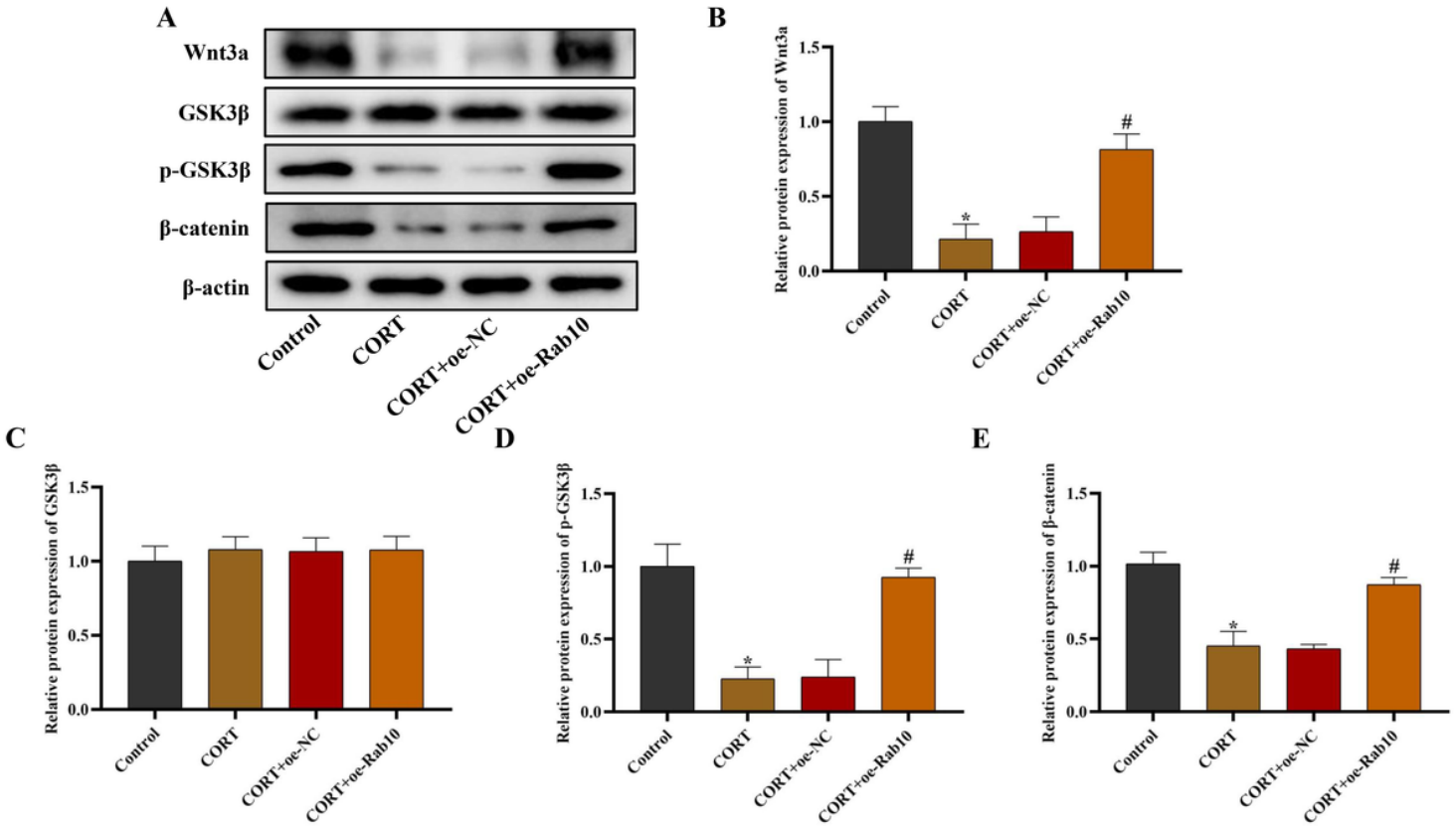

Figure 4

The effects of overexpressing Rab10 in cells on the Wnt/β-catenin signaling pathway by western blotting. A-E Immunoblots and quantitation of Wnt3a, GSK3β, p-GSK3β, and β-catenin protein expressions normalized to β-actin in PC12 cells after treatment with oe-Rab10, when examined by western blot analyses. $^{*}P < 0.05$ vs. the control. $^{\#}P < 0.05$ vs. CORT+oe-NC.

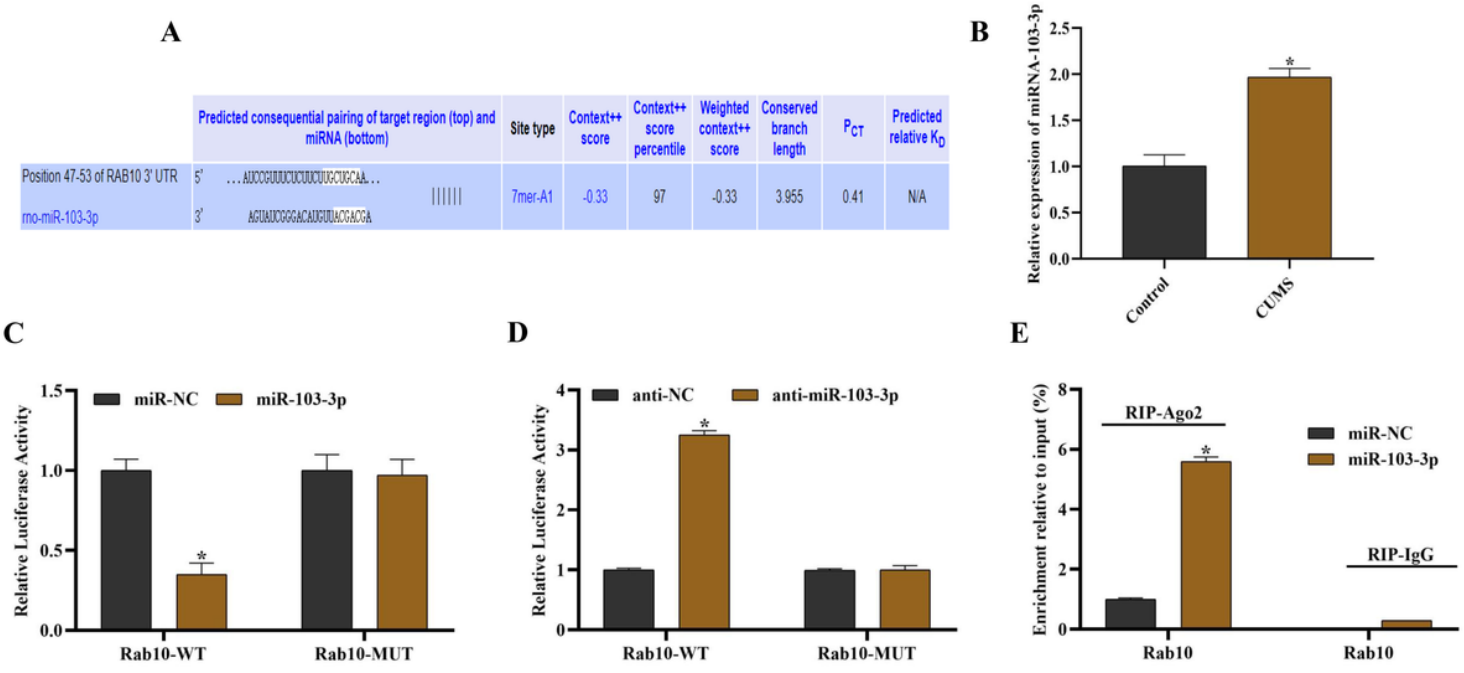

Figure 5

Validation of miRNA-103-3p association with Rab10. A The binding site of Rab10 was targeted by miRNA-103-3p in the TargetScan database. B Q-PCR validation of miRNA-103-3p expression in hippocampal tissue. C and D A double luciferase reporter experiment confirmed the interaction between miRNA-103-3p and Rab10. E The correlation between miRNA-103-3p and Rab10 was detected by the anti-Ago2 RIP method. $^{*}P < 0.05$ vs. the control, miR-NC, or anti-NC.

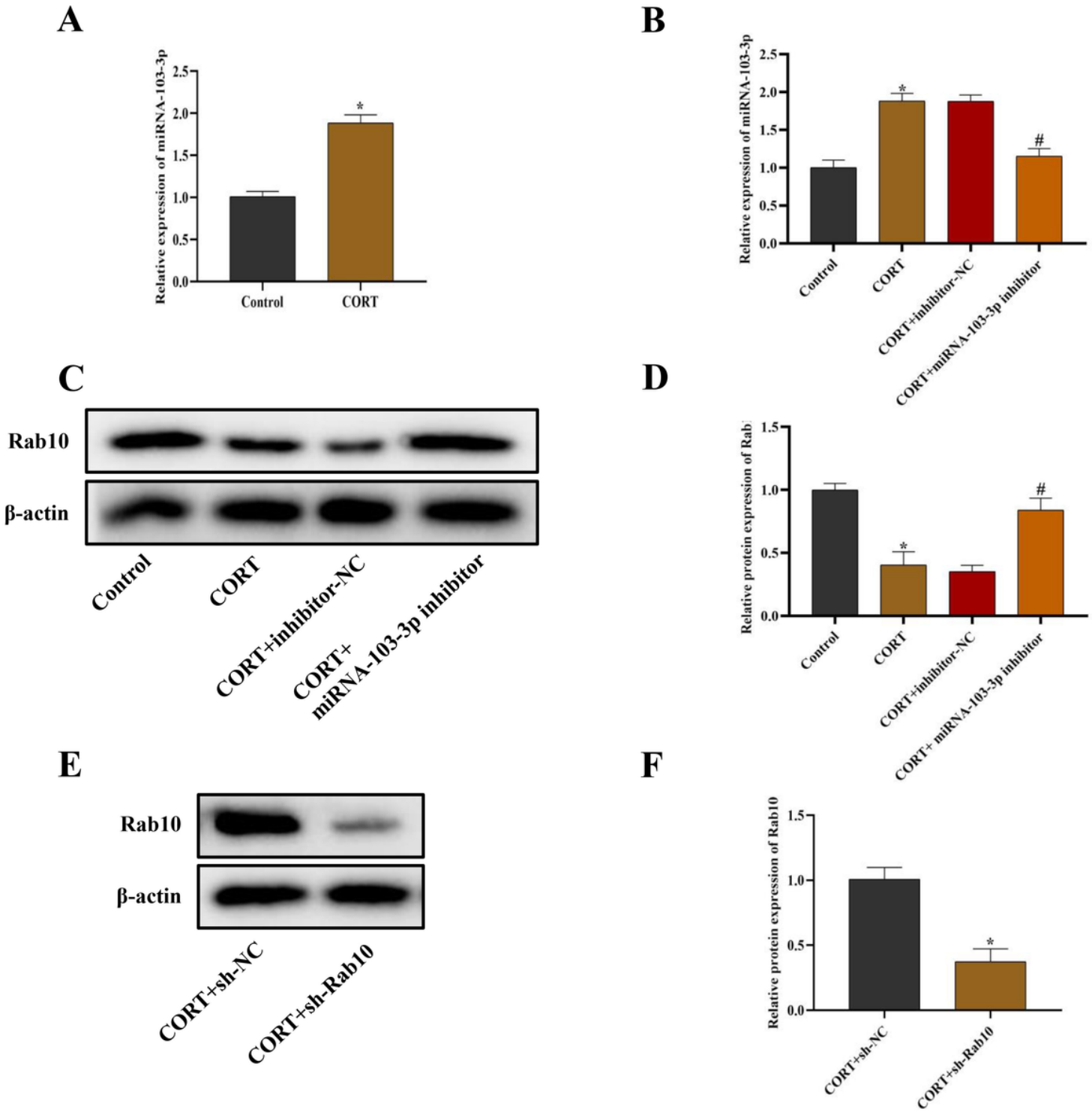

Figure 6

Effect of overexpressing miRNA-103-3p on Rab10 protein expression. A The level of miRNA-103-3p in PC12 cells induced by CORT was detected by Q-PCR. B The interference efficiency of miRNA-103-3p in PC12 cells was detected by Q-PCR. C Immunoblots of Rab10 and β-actin in Control, CORT, CORT+inhibitor-NC, and CORT+miRNA-103-3p inhibitor groups by western blot. D Rab10 statistical chart in Control, CORT, CORT+inhibitor-NC, and CORT+miRNA-103-3p inhibitor groups. E Immunoblots of Rab10 and β-actin in CORT+sh-NC and CORT+sh-Rab10 groups by western blot. F Rab10 statistical chart in

CORT+sh-NC and CORT+sh-Rab10 groups. *$P < 0.05$ vs. the control or CORT+sh-NC. #$P < 0.05$ vs. CORT+inhibitor-NC.

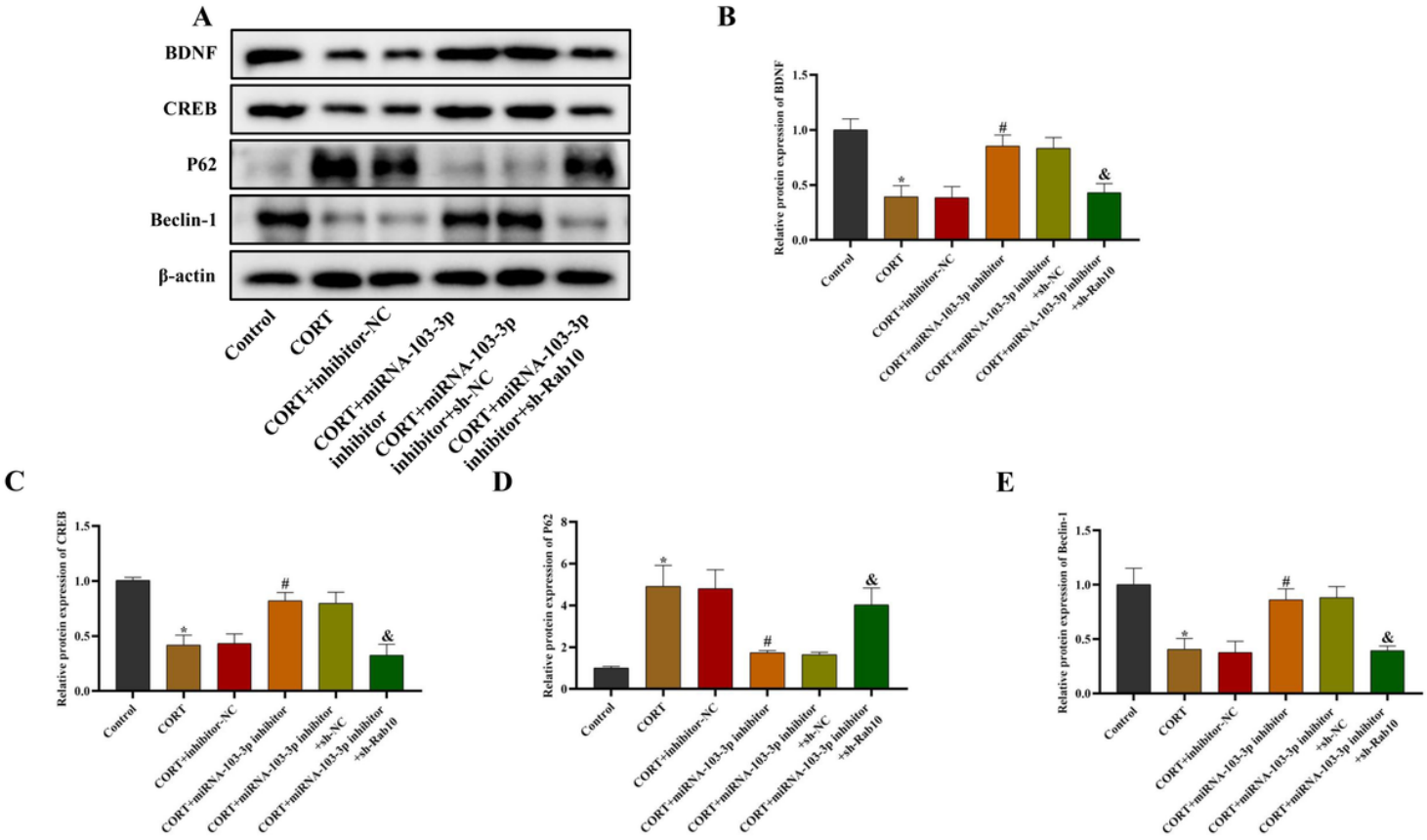

Figure 7

The influence of Rab10 regulation by miRNA-103-3p on the expressions of neuroplasticity and autophagy-related proteins. A-E Immunoblots and quantitation of BDNF, CREB, P62, and Beclin-1 protein expressions normalized to β-actin in PC12 cells measured by western blot analyses. *$P < 0.05$ vs. the control. #$P < 0.05$ vs. CORT+inhibitor-NC. &$P < 0.05$ vs. CORT+miRNA-103-3p inhibitor+sh-NC.

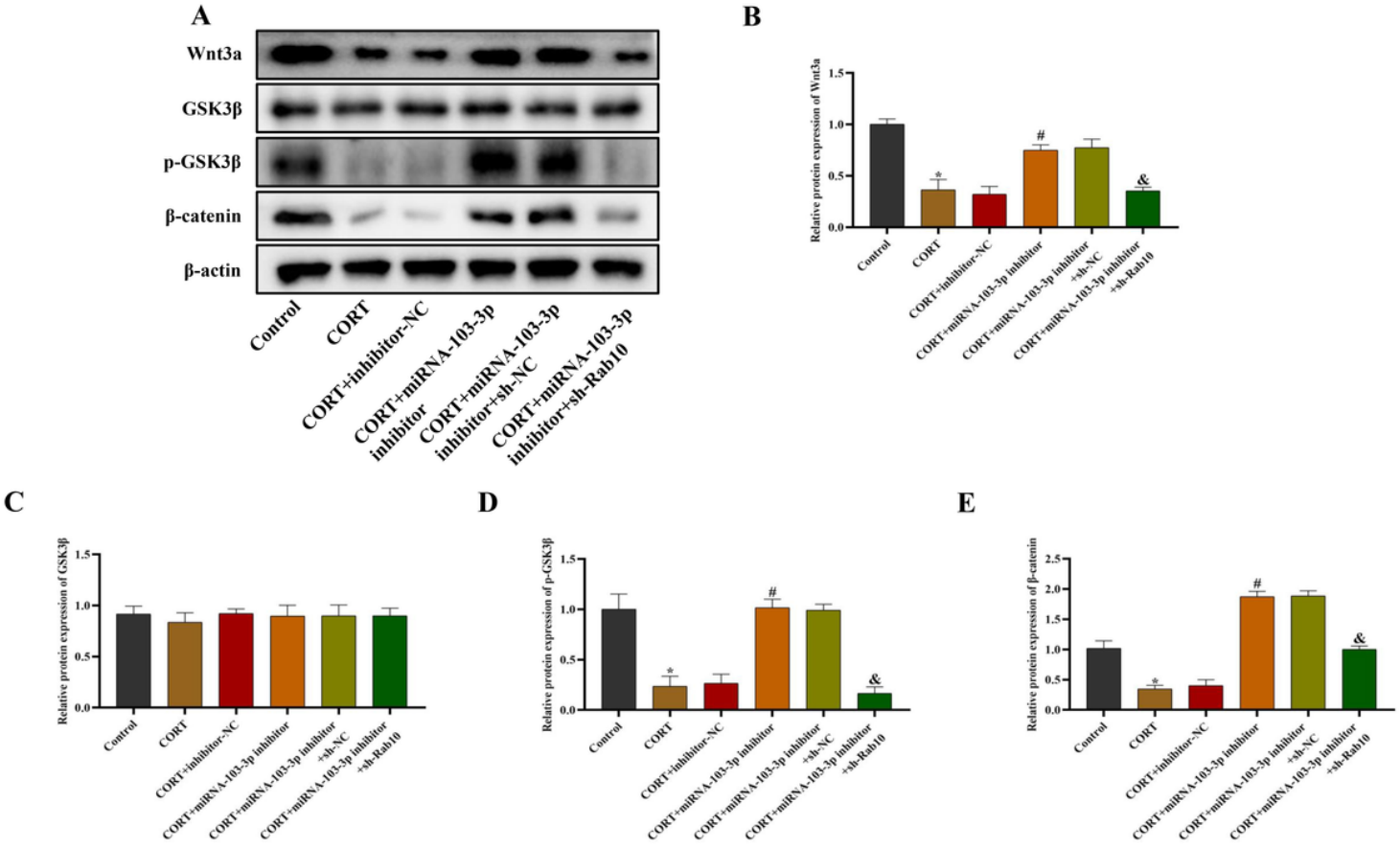

Figure 8

The effects of Rab10 regulation by miRNA-103-3p on the Wnt/β-catenin signaling pathway. A-E Immunoblots and quantitation of Wnt3a, GSK3β, p-GSK3β, and β-catenin protein expressions normalized to β-actin in PC12 cells measured by western blot analysis. $^{*}P < 0.05$ vs. the control. $^{\#}P < 0.05$ vs. CORT+inhibitor-NC. $^{\&}P < 0.05$ vs. CORT+miRNA-103-3p inhibitor+sh-NC.