Construction of SH-SY5Y cell line overexpressing Coronin 1a

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Abstract

Coronin 1a (also called CORO1A) is one of the members of Coronin family that regulate actin filament dynamics and cargo internalization,and involves Rac1/Pak1/Gpm6a signaling pathway to regulate synaptic plasticity. SH-SY5Y cells overexpressing CORO1A was constructed in this study. It showed that 12.5 μL/mL DNA-EndoFectin™ complex was the optimal concentration for infection of CORO1A lentiviruses. The results of fluorescence microscopy showed a successful construction of SH-SY5Y cell line overexpressing CORO1A.

Keywords

Coronin 1a, Overexpression, SH-SY5Y cell.

1. Introduction

Coronin family members have been described to play important roles in regulatingactin filament dynamics and cargo internalization [1]. They promote the formation of the leading edge of the migrating cells, contribute to the remodeling of actin during the formation of cell junctions, and participate in the endocytosis and endocytosis [2]. The Coronin family members are structurally similar and contain a WD40-repeat containing beta-propeller surface capable of binding F-actin [3]. In the study of coronins-GFP green fluorescent protein and knockout mutants, it has found that coronins participate in actin cytoskeleton rearrangement process relating to phagocytosis and cytosolic function. Coronin 1a (also called CORO1A)is located in cytoplasm and induces polarization of T cells induced by TCR-CD3. Thus it is accumulated on the F-actin-enriched membrane.CORO1A is associated with membrane motion and actin cytoskeleton [4]. It is important tonote that, CORO1A as an important intracellular second messenger can activate Gpm6a (M6a), through theRac1/Pak1/Gpm6a signaling pathway to regulatesynaptic plasticity. Therefore, SH-SY5Y cell line overexpressingCORO1A was constructed for furtherstudy on the regulation of synaptic plasticity in this study.

2. Experimental Detail

2.1 Materials

DMEM F12 medium, fetal bovine serum were purchased from Life Technologies. EndoFectinTM Max Expression Packaging Kitwere purchased from GeneCopeia.CORO1Amouse antibody was purchased from abcam. SH-SY5Ycells werepurchased from Clontech.

Reagent formulation

LB medium

Chemicals	Amount
Yeast Extract	5.0 g
Typton	10.0 g
NaCl	5.0 g
Ultra-pure water volume to	1 L

If the solid medium is added agar powder 15 g, 121°Cautoclave 20min, stored at 4°C.

Electrophoresis buffer

Chemicals	Amount
Tris-base	3.03 g
SDS	1.0 g
Gly	4.4 g
Ultra-pure water volume to	1 L

Transfer membrane buffer

Chemicals	Amount
Tris-base	5.8 g
SDS	0.37 g
Gly	2.9 g
Methanol	200 mL
Ultra-pure water volume to	1 L

2.2 Experimental Procedure

The total RNA was reverse transcribed to cDNA, PCR amplification, 1% agarose electrophoresis identification, the target band which was consistent with the expected size in the 1400 bp position. The target gene and pCMV-N-Flag vector were cut into two enzymes to connect and convert the DH5α of the Escherichia coli. The single colony on the Kna+ resistant plate was identified by double enzyme digestion. The results showed that all the monoclonal antibodies were positive, and there was a single, clear strip in the 1400 bp position. After the sequencing was accomplish, the plasmid was extracted without endotoxin and then transfected.

Primer sequence:

Sense:5'-GGAAGATCTATGAGCCGGCAGGTGGTTCG-3' ; Tm=61°C

Anti-sense:5'-CCGGAATTCCTACTTGGCCTGAACAGTCTCCTCCAG-3'; Tm=61°C

Reaction system:

Reaction component	Amount
Primer STAR (Premix)	25 μL
Primer F	2 μL (10 μM)
Primer R	2 μL (10 μM)
Template cDNA	2 μL
RNase-free Water	19 μL
Total Volume	50 μL

Reaction procedure:

Course of Reaction	Time	Temperature
Predenaturation	1 min	98°C
Denaturation	10 s	98°C
Annealing	10 s	55°C
Extend	10 s	72°C

2.3 pCMV-N-Flag Transformation and Amplification

A. DH5α competent cells were removed from -80°C in advance and thawed on ice

B. 1 μL pCMV-N-Flag plasmid was added to 50 μL receptive cells, mixed evenly with ice bath for 30 min.

C. Heat shock 90 s, then quickly transfer to ice, ice bath 2 min.

D. 400 μL LB medium (no resistance) was added to the incubator at 37°C concussion, 200 rpm/min, and concussionfor1 h.

E. 4000 × g was centrifuged for 5 mins, 400 L supernatant was discarded, then suspended with residual supernatant, and evenly coated in LB plate containing Kna+, fully absorbed for 10 min.

F. Culture for 12-16 h in flat plate inverted 37°C incubator.

G. Monoclonal culture was carried out with a small aseptic muzzle and cultured in LB liquid medium containing Kna+ for 12-16 h.

2.4 Transient Transfection ofSH-SY5Y Cells

A. Objective cell plank culture, The cells were digested with trypsin on the day before transfection and counted, and the cells were cultured on the pasteboard. On the second day, when the cell confluence was 70 ~ 80%, the transfection operation was carried out.

B. The DNA plasmid, EndoFectinTM Maxand transfection reagent was taken from the refrigerator and the culture medium was kept incubating to room temperature. DNA plasmids were diluted in Opti-MEM ITMmedium, EndoFectinTM Maxand transfection reagents were diluted. After that, the diluted DNA were gently mixed with the diluted EndoFectinTM Max transfection reagent. At room temperature, the EndoFectinTM Max complex was fully formed by standing at 5°C for 20 min.

C. The DNA-EndoFectinTM complex was added to the culture plate hole one by one, and the culture plate was gently shockedfor mixwholly.

D. The cells were incubated in CO2 incubator at 37°C for 24 h.

Fluorescence intensity was observed by fluorescence microscopy, and the protein expression of CORO1Awas identified by Western blotting.

2.5 Statistical Analysis

One-way ANOVA followed by Bonferroni post hoc test was used for data analyses with statistics software (SPSS 19.0). All experimental data represent means ± SEM, and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Construction of Expression Vector

The total RNA was extracted by reverse transcription into cDNA and identified by PCR amplification with 1% agarose gel electrophoresis. A target band of CORO1A was found at the position of 1400bp, which was consistent with the expected size. After the pCMV-N-Flagvector were digested by double enzyme, the target gene was ligated and transformed into the competent DH5α, and the single colony on theKna+ resistant plate was selected for double enzyme digestion. Results as shown in the figure 1, the identified monoclonal was positive, with a single and distinct band at 1400 bp (Fig. 1). After sequencing successfully, the plasmids without endotoxin can be extracted and then transfected.

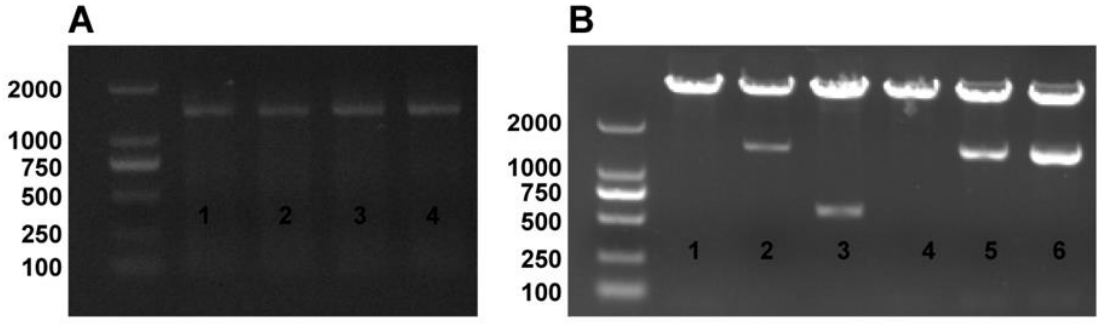

Fig 1. Construction of recombinant vector (A) 1, 2, 3, 4: CORO1A gene amplification; (B) 1, 2, 3, 4, 5, 6: restriction enzyme digestion results of positive monoclonal. The 6th sample was sent to detect the complete sequence.

3.2 Construction of Overexpression Coro1a in SH-SY5Y Cell Line

The concentration of EndoFectinTM Max transfection reagent and transfection time were explored. The EndoFectin TM Max transfection reagent was diluted into a DNA-EndoFectinTM complex with a working concentration of 5 μL/mL, 7.5 μL/mL, 12.5 μL/mL on Opti medium. The DNA-EndoFectinTM complex was transfected into SH-SY5Y cells and the expression of CORO1A protein was detected by Western blotting. The results showed that (Fig. 2), the expression of CORO1A protein in the cells transfected with 12.5 μL/mL was significantly higher than that in the control group, and the best time was 24 h after transfection, compared with the control group. Sothis concentration and transfection time are chosen as the optimal condition.

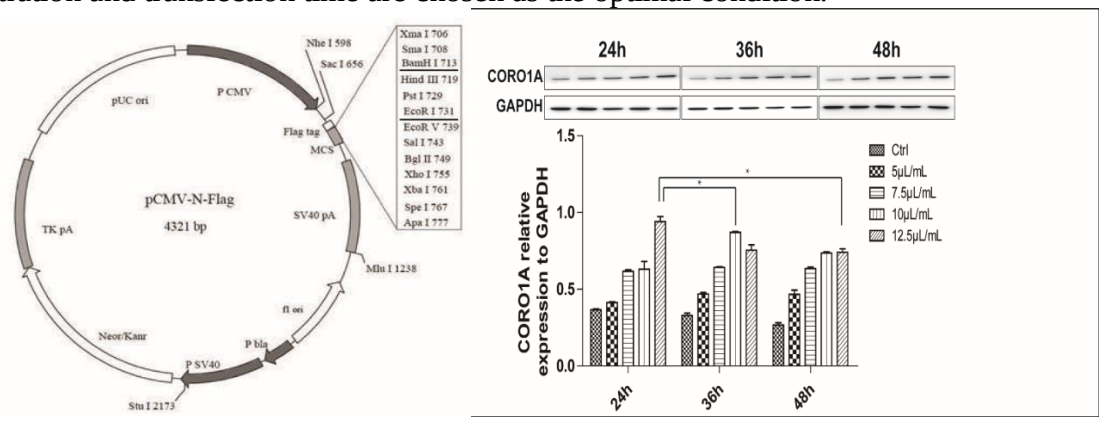

Fig 2. Protein expression of CORO1A in SH-SY5Y cells transfected with different concentration and time.

3.3 Identification of CORO1Aoverexpression in SH-SY5Y cells

The results of laser confocal immunofluorescence showed (Fig. 3) that compared with the control group, the process growth of CORO1A overexpression cell line and the average protruding length of the over-expressed cell line was longer than that of the control group.

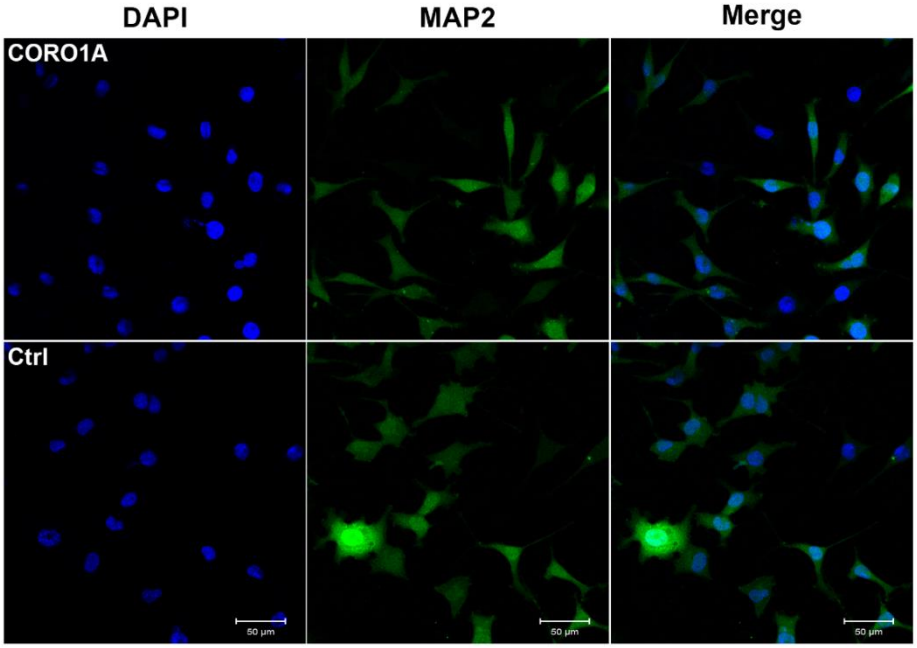

Fig 3. Identification of CORO1A overexpression in SH-SY5Y cells by Immunofluorescence

4. Conclusion

In this study, we found that the ratio of 12.5 μL/mL DNA-EndoFectinTM complexwas the optimal concentration ratio for transfection of CORO1A vector. The results of fluorescencemicroscopy and Western blotting showed a successful construction of SH-SY5Y cell line overexpressingCORO1A.

Acknowledgements

The work was supported by grants of the National Natural Science Foundation of China (No.31271607).

The work was supported by Medical Scientific Research Foundation of Guangdong Province, China(A2016469).

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