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Analysis of Signaling Pathway for the Differentiation of Neural Stem Cells

Wei Lu[1] Jiao Wang[1,2] Tieqiao Wen[1,*]

[1] Institute of Systems Biology, Shanghai University, 200444, China
[2] School of Life Sciences, Shanghai University, 200444, China

Abstract G13 signaling pathway is closely related with differentiation of neural stem cells. Rho-GDIγ is a key factor in this pathway. When Rho-GDIγ is silenced by using RNAi, the neural stem cell appears distinguished morphological change. The results indicate that the silenced cells showed the tendency of stimulative differentiation of neural stem cells.

1 INTRODUCTION

Neural stem cells (NSCs) are the cells that can differentiate into neurons, astrocytes, and oligodendrocytes, and also sufficiently provide the numbers of cells by self-renewing in the brain. Rho proteins belong to the small GTP-binding family G Proteins, which consist of the Rho, Rac, and Cdc42 subfamilies. Rho family members regulate various cell functions through reorganization of the actin cytoskeleton, such as cell shape change, cell aggregation, cell-to-cell adhesion, membrane ruffling, formation of stress fibers and focal adhesions, cell motility, cytokinesis, and smooth muscle contraction. The Rho-GDI family of proteins (α, β, and γ) is one of three central regulators of the RhoA/Rac/Cdc42 family of small GTPases. So far, three Rho-GDIs have been identified. Rho-GDIα is first cloned from brain and is found ubiquitously expressed. Rho-GDIβ is found preferentially expressed in hematopoietic cells. Rho-GDIγ was cloned from a whole embryo library and is found expressed at high levels in only 2 tissues, brain and pancreas [1].

Recent studies have shown that RNAi technique was successfully applied to neuroscience studies. Effective gene silencing using RNAi has been demonstrated in neuronal cell lines [2], neural stem cells [3], primary mammalian neurons [4], astrocytes [5] and Schwann cells [6].

Our previous research of the differentiation of neural stem cells through microarray detected that over 10 thousands genes were expressed during this process [7]. So in this report we deduce the pathway of gene expression and regulation by bioinformatics method and indicate a crucial role of Rho-GDIγ gene in the differentiation of neural stem cells.

*Corresponding author. E-mail address: tqwen@staff.shu.edu.cn.

2 RESULTS

Rho-GDIγ is a key regulator

Gene expression was quantified in differentiating neural stem cells using microarray and the data were analyzed through Bioinformatics method. The result of the bioinformatics analysis is shown in Fig. 1. It can be seen that Rho-GDIγ is related several different proteins. This suggests that Rho-GDIγ may play a crucial role in regulating differentiation of neural stem cells.

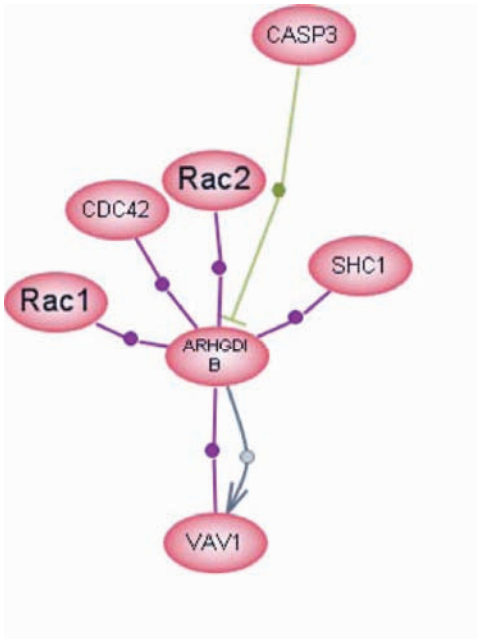
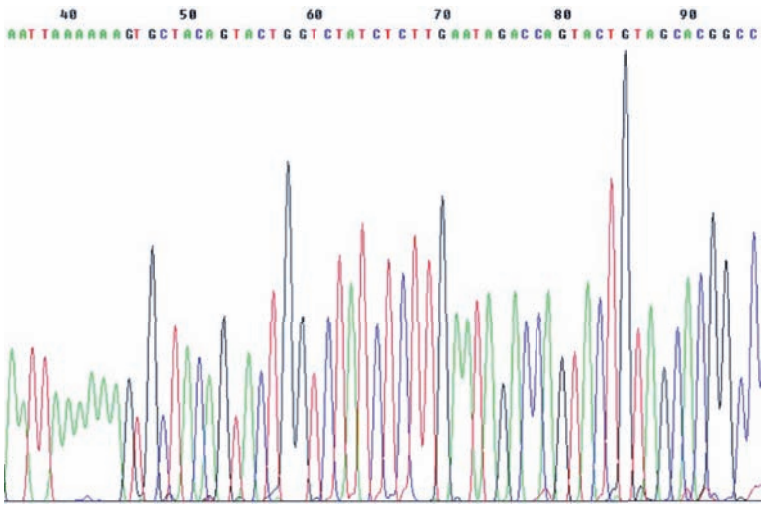


Figure 1: Analysis of interactive proteins among Rho GDI

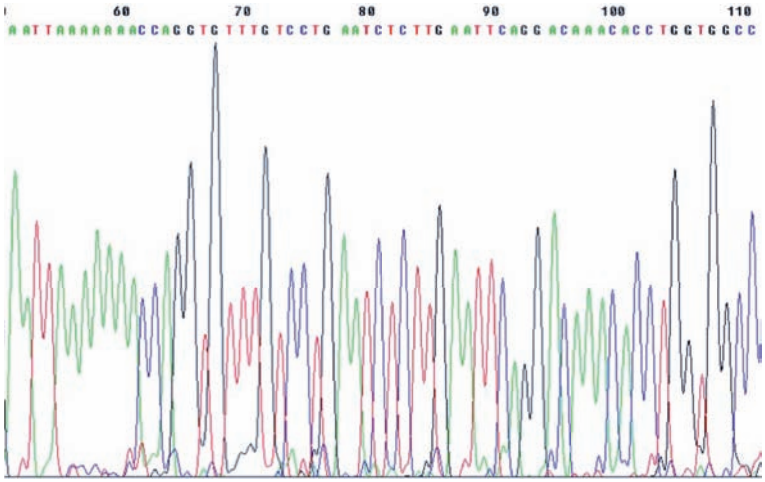
Effect of Rho-GDIγ-targeted RNAi on differentiation of neural cells

RNAi-expressing plasmids were constructed to determine the effect of down-regulating Rho-GDIγ in neural cells. The sequences of the RNAi-coding insert and plasmid construct are shown in Figure 2.

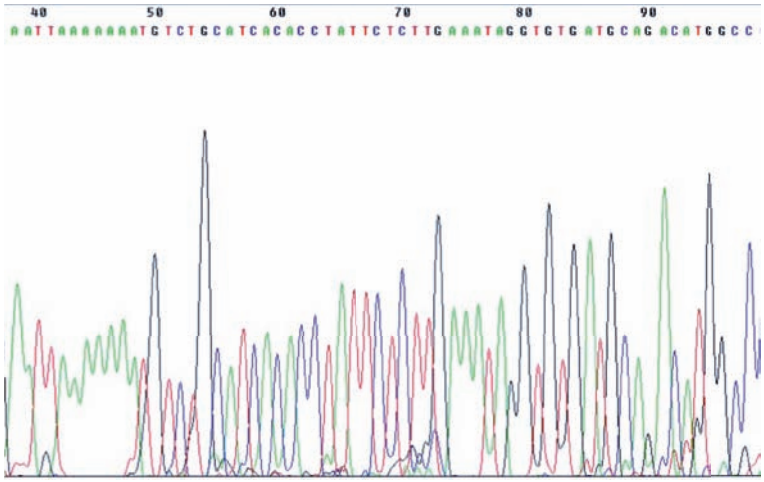
One week after the cells were transfected with RNAi constructs, cellular shape were observed under the Nikon inverted microscope. The result shows that cells expressing the control RNAi construct were regular in shape. In contrast, cells expressing Rho-GDIγ-targeted RNAi had long neurites and altered cell shape (Fig.3). This result suggests that downregulation of Rho-GDIγ promotes differentiation of neural stem cells and that Rho-GDIγ may be a negative regulator of neural differentiation.



(a)



(b)



(c)

Figure 2: Sequencing of RNAi silence sequences. A is control sequence; B and C are the silence sequences respectively.

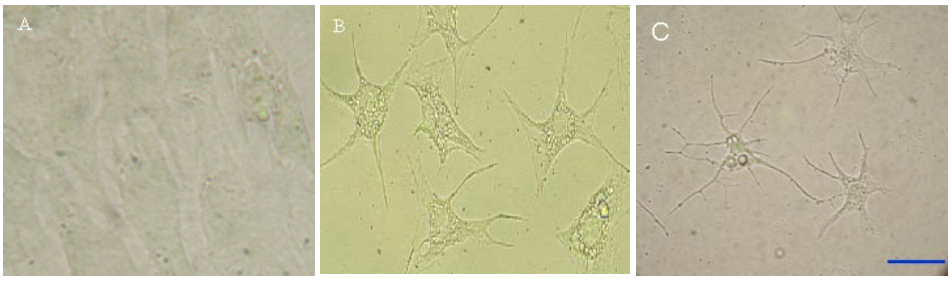


Figure 3: Effect of Rho-GDI γ-targeted RNAi on differentiation of neural stem cells. A, B and C are control sequence; silence sequences respectively.

Behavior test after Rho-GDIγ silenced

Mice behaviors were analyzed after in vivo transfection with RNAi vector. There were almost no differences from the data of gaining of weight (g/day) and stand times/min; While the data of horizontal movement and the duration time of swimming showed significant differences (**p<0.01) after in vivo silence of Rho-GDIγ (Fig.4).

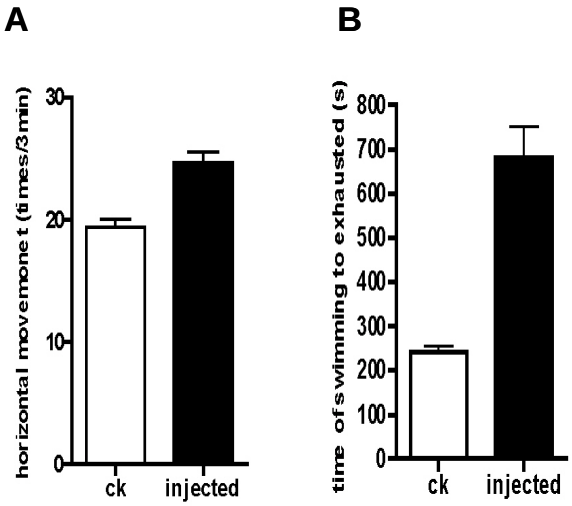


Figure 4: Statistical analysis of behavior. *Ck* was for the control group injected with the plasmid solution of Ck*; Injected* was for the group injected with the plasmid solution of RNAi silences. The data of horizontal movement (times/3min) (A) and duration time of swimming to exhausted (B) showed significantly differences (**p<0.01).

3 Materials and methods

Bioinformatics analysis

Bioinformatics analysis of interactive proteins was performed through PathwayStudio 4.0 software.

Design and construction of RNAi-expressing plasmids

The RNAi sequence and structure was determined using the Ambion "siRNA Target Finder and Design Tool". Typically, siRNA target sites were chosen by scanning an mRNA sequence for AA dinucleotides, recording the 19 nucleotides immediately downstream of the AA, and then comparing the potential siRNA target sequences with an appropriate genome database to ensure the absence of significant homology to other genes. When expressed in vivo, the insert forms a stem-loop hairpin structure.

Cell culture

Mouse neural stem cell line C17.2, which was kindly provided by Dr Evan Y Snyder, was cultured as previously described [8] in high-glucose DMEM with 10% fetal bovine serum (Sigma), 5% horse serum (Gibco), 1mM glutamine, 100 U/ml penicillin and 100 μ?/ml streptomycin, culture dishes in standard humidified 5% CO2 at 37˚C. Cells were maintained in culture either by splitting1:5 into fresh medium .Cells were fed with fresh medium twice weekly and passaged once per week.

Acknowledgment

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