RALY participates in nerve trauma-induced nociceptive hypersensitivity through triggering eIF4G2 gene expression in primary sensory neurons

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Abstract

Background and Purpose: Peripheral nerve trauma-induced dysregulation of pain-associated genes in the primary sensory neurons of dorsal root ganglion (DRG) contributes to neuropathic pain genesis. RNA-binding proteins participate in gene transcription. We hypothesized that RALY, an RNA-binding protein, participated in nerve trauma-induced dysregulation of DRG pain-associated genes and nociceptive hypersensitivity. Methods and results: Immunohistochemistry staining showed that RALY was expressed exclusively in the nuclei of DRG neurons. Peripheral nerve trauma caused by chronic constriction injury (CCI) of unilateral sciatic nerve produced time-dependent increases in the levels of Raly mRNA and RALY protein in injured DRG. Blocking this increase through DRG microinjection of adeno-associated virus 5 (AAV5)-expressing Raly shRNA reduced the CCI-induced elevation in the amount of eukaryotic initiation factor 4 gamma 2 (eIF4G2) mRNA and eIF4G2 protein in injured DRG and mitigated the development and maintenance of CCI-induced nociceptive hypersensitivity, without altering basal (acute) response to noxious stimuli and locomotor activity. Mimicking DRG increased RALY through DRG microinjection of AAV5 expressing Raly mRNA upregulated the expression of eIF4G2 mRNA and eIF4G2 protein in the DRG and led to hypersensitive responses to noxious stimuli in the absence of nerve trauma. Mechanistically, CCI promoted the binding of RALY to the promoter of eIF4G2 gene and triggered its transcriptional activity. Conclusion and Implications: Our findings indicate that RALY participates in nerve trauma-induced nociceptive hypersensitivity likely through transcriptionally triggering eIF4G2 expression in the DRG. RALY may be a potential target in neuropathic pain management.

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Fig. 1

A. Relative level of Raly mRNA

B. Days after CCI or sham surgery

C. Days after CCI or sham surgery

D. Relative level of Raly protein

Sham CCI

Days 0 3 7 14

Relative level of RALY protein

Days after CCI or sham surgery

Days after CCI or sham surgery

Con L3/4 DRGs Ipsi L3/4 SC

Relative level of RALY protein

Days after CCI

Sham SNL

Relative level of RALY protein

Days after CCI
Fig. 2

A. RALY/β-tubulin-III

B. RALY/γS/DAPI

C. Cross-sectional area (µm²)

D. RALY/CGRP

E. RALY/IB4

F. RALY/NF200

G. Sham

H. Percentage of RALY positive neurons/section (%)

* Indicates significant difference between Sham and CCI groups.
Fig. 5

A) Paw withdrawal frequency (%)
B) Paw withdrawal frequency (%)
C) Paw withdrawal latency (s)
D) Paw withdrawal latency (s)

E) Relative level of RALY protein
F) Relative level of proteins

CCI: Contralateral Commissure Injury
 ICC: Ipsilateral Commissure Injury

** indicates significance at p < 0.05.
Fig. 8

A

Sham + Scr  CCI + Scr  CCI + shRNA

B

Relative level of eIF4g2  expression

C

Sham + Scr  CCI + Scr  Sham + shRNA

D

Relative level of eIF4g2 mRNA

E

Relative level of eIF4g2 protein

F

Relative level of eIF4g2 mRNA

G

Relative level of eIF4g2 protein

H

Relative level of Raly mRNA  eIF4g2 mRNA

I

Large  Medium  Small

Raly  Eif4g2  Gapdh  NeuN