NEURAL REGENERATION RESEARCH
Volume 7, Issue 13, May 2012
www.nrronline.org

Cite this article as: Neural Regen Res. 2012;7(13):965-970.

Actin-binding Rho activating protein is expressed in the central nervous system of normal adult rats★

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Abstract

Previous studies show that actin-binding Rho activating protein (Abra) is expressed in cardiomyocytes and vascular smooth muscle cells. In this study, we investigated the expression profile of Abra in the central nervous system of normal adult rats by confocal immunofluorescence. Results showed that Abra immunostaining was located in neuronal nuclei, cytoplasm and processes in the central nervous system, with the strongest staining in the nuclei; in the cerebral cortex, Abra positive neuronal bodies and processes were distributed in six cortical layers including molecular layer, external granular layer, external pyramidal layer, internal granular layer, internal pyramidal layer and polymorphic layer; in the hippocampus, the cell bodies of Abra positive neurons were distributed evenly in pyramidal layer and granular layer, with positive processes in molecular layer and orien layer; in the cerebellar cortex, Abra staining showed the positive neuronal cell bodies in Purkinje cell layer and granular layer and positive processes in molecular layer; in the spinal cord, Abra-immunopositive products covered the whole gray matter and white matter; co-localization studies showed that Abra was co-stained with F-actin in neuronal cytoplasm and processes, but weakly in the nuclei. In addition, in the hippocampus, Abra was co-stained with F-actin only in neuronal processes, but not in the cell body. This study for the first time presents a comprehensive overview of Abra expression in the central nervous system, providing insights for further investigating the role of Abra in the mature central nervous system.

Key Words

actin-binding Rho activating protein; actin cytoskeleton; confocal immunofluorescence; striated muscle; nervous tissue; neural regeneration

Abbreviations

Abra: actin-binding Rho activating protein; ABP: actin-binding protein; CNS: central nervous system

INTRODUCTION

Actin-binding Rho activating protein (Abra) (also known as STARS), a novel and evolutionarily conserved actin-binding protein (ABP), binds to the I-band of the sarcomere and to actin filaments in transfected cells, where it activates Rho-signaling events[1]. Abra stimulates SRF-dependent transcriptional activity through a mechanism that requires actin binding and involves Rho GTPase activation. In cardiac tissue, Abra expression is upregulated in mouse models of cardiac hypertrophy and in failing human hearts, and Abra modulates the responsiveness of the heart to stress signaling by functioning

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Received: 2012-03-02
Accepted: 2012-04-07
(N20120424009/H)

Liu LH, Luo MY, Yang BL, Wu XQ, Zhu W, Guan YL, Cai WJ, Troidl K, Schaper W, Schaper J. Actin-binding Rho activating protein is expressed in the central nervous system of normal adult rats. Neural Regen Res. 2012;7(13):965-970.

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doi:10.3969/j.issn.1673-5374.2012.13.001

as a cytoskeletal intermediary between MEF2 and SRF[2-3]. Recently, Abra signaling pathway has been shown to be responsive to changes in skeletal muscle loading and appears to play a role in both human skeletal muscle hypertrophy and atrophy[4]. Evidence exists that Abra is highly upregulated in growing collaterals and leads to stimulation of collateral growth by smooth muscle cell proliferation[5-6]. These studies have shown that Abra is involved in regulating actin cytoskeletal function and actin polymerization in various cells.

Like in muscle cells[7], actin is also one of most abundant proteins in neurons. Numerous findings demonstrated that actin cytoskeleton plays a pivotal role in dendritic spine morphology[7-9] and the motility of the growth cone[10], and their activities are regulated by a wide variety of ABPs like Arp2/3[11-12], ADF/cofilin[13-14] and fascin[15-17]. Corresponding to the expression of actin in muscle and nerve cells, those ABPs are expressed in nerve cells, most also in muscle cells, such as profilin[18], cofilin, gelsolin[19-20], tropomysin[21] and fascin.

Based on the information cited above, we hypothesized that Abra as a novel ABP expressed in the striated muscles and vascular smooth muscle cells could also be expressed in nervous tissue. To test this hypothesis, we examined the expression profiles of Abra in the central nervous system (CNS) of normal adult rat by confocal immunofluorescence with specific antibodies.

RESULTS

Wide expression of Abra in the cerebral cortex

In the cerebral cortex, Abra-immunopositive neuronal bodies and processes were distributed widely in six cortical layers, including the molecular layer, external granular layer, external pyramidal layer, internal granular layer, internal pyramidal layer and polymorphic layer (Figures 1A–A″). Among the total cell number (4', 6-diamidino-2-phenylindole (DAPI) stained nuclei as determined by confocal microscopy on randomly selected cells) in the cerebral cortex, a major proportion (approximately 64.1 ± 4.0%) was immunostained with Abra antibody. Abra immuostaining was located in neuronal nucleus, cytoplasm and processes. The strongest Abra staining was located at the neuronal nucleus (Figures 1B–B″). Abra and F-actin double immunostaining showed that F-actin was distributed in the neuronal body and their processes, and co-localized with Abra in neuronal cytoplasm and processes (Figures 1C–C″). There was difference in Abra expression in the cerebral cortex between males and females (data not shown).

Expression of Abra in the hippocampus

Abra-immunopositive cells were distributed evenly in the hippocampal subiculum, CA1, CA2, CA3 and dentate gyrus (Figure 2).

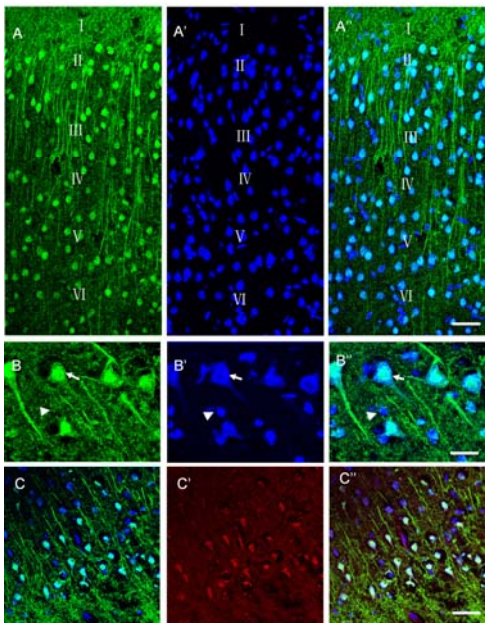

Figure 1 Confocal micrographs of actin-binding Rho activating protein (Abra) immunostaining in the cerebral cortex. Numerous Abra-positive neuronal bodies and processes (green) are distributed widely in layers I–VI of the cerebral cortex.

Positive cells are mainly present in layers II, III, V and VI, only a few are scattered in layer IV (A–A″). Abra immuostaining was located in the neuronal nucleus, cytoplasm and processes. The strongest staining of Abra is in the neuronal nucleus (B–B″).

Double immunostaining showed the co-localization of Abra and F-actin (red) in neuronal cytoplasm and processes (C–C″). Nuclei are stained with 4',6-diamidino-2-phenylindole (blue). Scale bars: 40 μm in A, C; 20 μm in B.

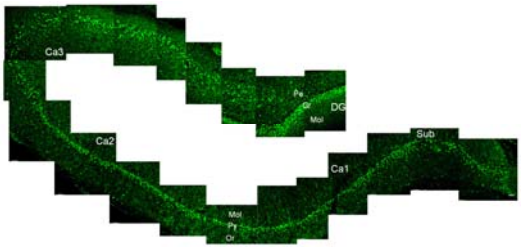

Figure 2 Confocal micrographs of Abra immunostaining in the hippocampus. Abra-immunopositive cells (green) are distributed evenly in all hippocampal areas.

Abra: Actin-binding Rho activating protein; Mol, molecular layer; Py, pyramidal layer; Or, oriens layer; Gr, granular layer; Pe, pexiform layer; Sub, subiculum; DG, dentate gyrus. Scale bars: 50 μm.

The Abra-positive cell bodies were widely located in pyramidal layer and granular layer, corresponding to

wide distribution of Abra-positive processes in molecular layer, oriens layer and pexiform layer (Figure 2). Cell counting showed that approximately 62.0 ± 4.0% of cells DAPI-stained nuclei as determined by confocal microscopy on randomly selected cells) could be labeled with Abra antibody in the pyramidal layer and granular layer. Abra immuostaining was located in neuronal nucleus, cytoplasm and processes. Double immunostaining of Abra and F-actin showed that Abra was co-localized with F-actin in the neuronal processes, not in the cell bodies (Figure 3).

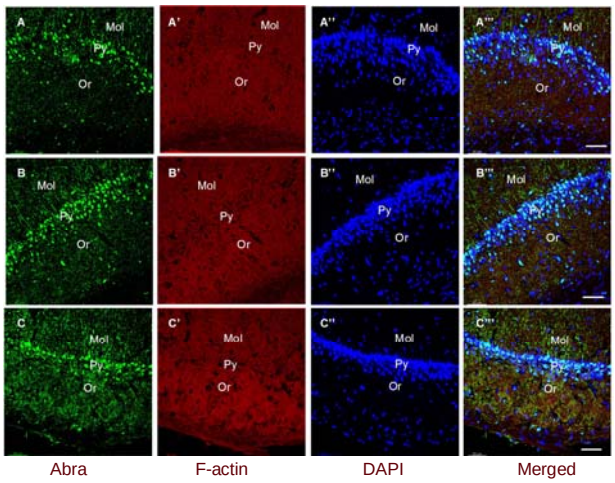

Figure 3 Double immunostaining of actin-binding Rho activating protein (Abra) and F-actin in the hippocampus. Abra (green) is co-localized with F-actin (red) in the neuronal processes, but not in the cell bodies at the areas subiculum (A–A″), CA1(B–B″), CA2 (C–C″).

Nuclei are marked by 4',6-diamidino-2-phenylindole (DAPI; blue). Or: Oriens layer; Mol: molecular layer; Py: pyramidal layer. Scale bars: 50 μm.

Expression of Abra in the basal ganglia

In the basal ganglia, Abra-immunopositive products were also observed in neuronal nucleus, cytoplasm and processes, and the strongest staining of Abra was in the neuronal nucleus (Figure 4).

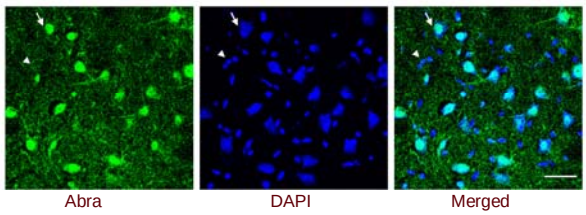

Figure 4 Confocal micrographs of actin-binding Rho activating protein (Abra) immunostaining in the basal ganglia. Abra-positive products (green) are observed in neuronal nucleus, cytoplasm and processes at the basal ganglia. Scale bar: 40 μm. DAPI: 4',6-diamidino-2-phenylindole.

Cell counting showed that the Abra-immunopositive cells accounted for approximately 52.1 ± 3.0%.

Abra expression in the cerebellar cortex

Numerous Abra-positive neuronal bodies and processes were distributed in the molecular layer (Mol), granular layer (Gr) and Purkinje cell layer (P) of the cerebellar cortex (Figure 5). Intensely stained piriform cells were regularly arranged along the Purkinje cell layer, with obvious Abra staining of the nuclei. Double immunostaining of Abra and F-actin showed that F-actin-expressing neurons and fibers were immunostained with Abra antibody (Figure 5). F-actin-positive products were distributed in the neuronal bodies and processes. Double immunostaining showed that Abra co-localized with F-actin in neuronal cytoplasm and processes.

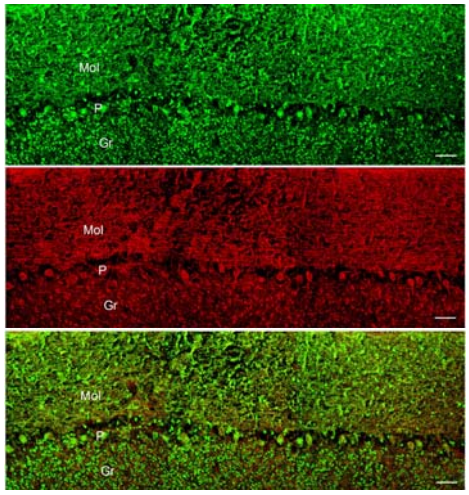

Figure 5 Double immunostaining of actin-binding Rho activating protein (Abra) and F-actin in the cerebellar cortex. The upper panel shows Abra staining (green), the middle one for F-actin staining (red), and the lower one is merged image.

Numerous Abra-positive neuronal bodies and processes are distributed in the molecular layer (Mol), granular layer (Gr) and Purkinje cell layer (P) of the cerebellar cortex.

Abra co-localized with F-actin in neuronal cytoplasm and processes. Mol: Molecular layer; Gr: granular layer; P: Purkinje cells layer. Scale bars: 50 μm.

Expression of Abra in the spinal cord

In the spinal cord, Abra was intensely expressed in the gray matter and white matter. Abra-positive products were distributed in all laminas of gray matter. Within the white matter, a more intense Abra staining could be detected in marginal regions than in the inner zone (Figures 6A–B″). Within the gray matter, Abra-positive neurons were mainly localized in outer part of lamina II, with a few additional cells distributed sparsely throughout the other laminae of the spinal dorsal horn and intermediate region, and Abra-positive fibers were present in all laminas of the spinal dorsal horn, intermediate region and anterior horn (Figures 6A–B″). In the lamina IX of anterior horn, some

large-sized motor neurons were intensely stained (Figures 6B–C″). Nucleus and the zone near the cell membrane of these large motor neurons were strongly stained (Figures 6C–C″). Abra-positive products were also found in the posterior root (Pr). Double immunostaining of Abra and F-actin showed that Abra partly co-localized with F-actin in the spinal cord (Figures 6A–C″). F-actin-positive products were detected in the neuronal body and processes, but none were found in the neuronal nucleus (Figures 6C–C″).

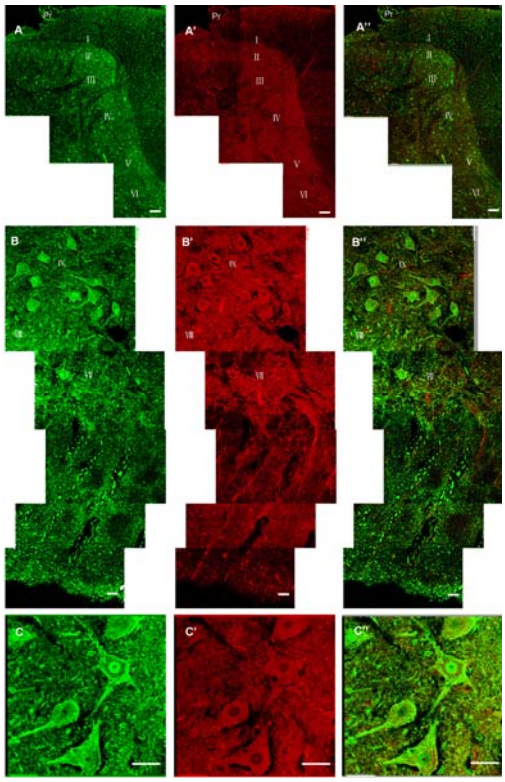

Figure 6 Double immunostaining of Abra (green) and F-actin (red) in the spinal cord. Abra was intensely expressed in the gray matter and white matter of the spinal cord. Abra: Actin-binding Rho activating protein; Pr: posterior root. Scale bars: 50 μm in A, B; 40 μm in C.

Abra-positive neurons are mainly localized in outer part of lamina II, with a few additional cells distributed sparsely throughout the other laminae of the spinal dorsal horn (A–A″) and intermediate region (A–B″).

Abra-positive fibers are present in all laminas of the spinal dorsal horn, intermediate region and anterior horn (A–B″).

In the lamina IX of anterior horn, some large-sized motor neurons are intensely stained (C–C″).

Identification of the specificity of anti-Abra antibody

When Abra antibody was pre-absorbed with the antigenic peptide, Abra immunofluorescence was hardly detected or significantly reduced depending on amounts of the antigenic peptide used (Figure 7). In addition, no Abra immunoreactivity was observed when Abra antibody was omitted or replaced with normal goat IgG. For positive control, previous observations of a partial overlap of the Z-line in longitudinal sections of skeletal muscle could be confirmed by co-immunostaining with anti-Abra and anti-α-actinin (Figure 8).

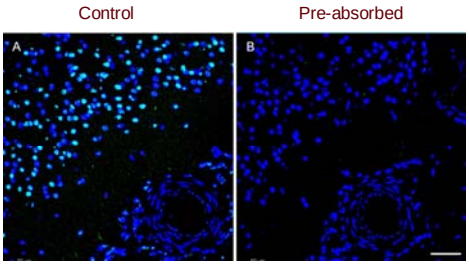

Figure 7 Confocal micrographs showing immunostaining of actin-binding Rho activating protein (Abra) with or without pre-absorption in serial sections. Specific fluorescence: green for Abra, blue for nuclei. Scale bar: 50 μm.

(A) Abra immunostaining without pre-absorption; (B) Abra antibody is pre-absorbed with antigenic peptide. Notice: Immunoreactivity of Abra is hardly detected in B.

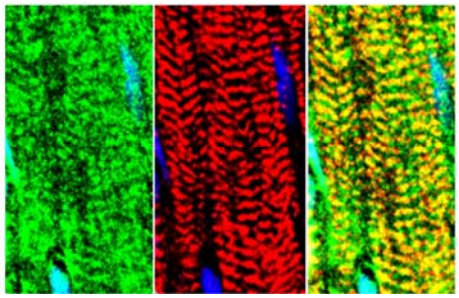

Figure 8 Confocal micrographs of actin-binding Rho activating protein (Abra) immunostaining in skeletal muscles. In longitudinal sections of skeletal muscles, Abra (green) and α-actinin (red) staining showed partial overlap of Abra with the Z-line. Scale bars: 40 μm.

DISCUSSION

In this study, the expression profile of Abra in the adult rat brain and spinal cord was examined by confocal immunofluorescence. Varying intensities of Abra-immunopositive products were distributed widely in the cerebral cortex, hippocampus, basal ganglia, cerebellum and spinal cord. Abra-positive staining was observed in neuronal nucleus, cytoplasm and processes, and the strongest staining of Abra was found in the neuronal nucleus. This data suggests that Abra may play a role in the central nervous system.

To the best of our knowledge, this is the first report that Abra, a novel and evolutionarily conserved actin-binding protein is expressed in nerve cells.

ABPs play an important role in the nerve cells including neuronal morphogenesis, spine and growth cone. Previous studies showed that Abra was involved in SRF

transcriptional activity, cardiac and skeletal muscle hypertrophy and smooth muscle cell proliferation in collateral vessels[2-3, 5-6]. However, proliferation does not occur in mature nerve cells, therefore, the role of widely expressed Abra in nerve cells needs to be further explored. F-actin is composed of long filamentous polymers containing two strands of globular monomers. The assembly, remodeling and disassembly of the actin filament are strictly regulated by a large number of ABPs[22-24]. To outline the relationship between Abra and F-actin, these two proteins were co-stained. We observed that F-actin was moderately stained in cell bodies and processes, weakly stained in the nuclei of nerve cells in most regions in the central nervous system except for the pyramidal layer in the hippocampus where F-actin was very weak, even negative in cell bodies and nuclei. This data is consistent with previous results [25]. The colocalization of Abra and F-actin in the cytoplasm and processes of the nerve cells is conceivable since ABP has essential roles in the regulation of F-actin organization. However, the fact that the presence of high level of Abra and very lower level of F-actin in the nuclei of the nerve cells is very interesting. It might imply that Abra not only participates in regulating actin function, but also in other functions as a nuclear protein. It remains to be determined.

In conclusion, this study for the first time demonstrates that Abra is a novel ABP in the nerve cells by presenting a comprehensive overview of Abra expression in the central nervous system. It may serve as a springboard for further mechanism studies of Abra in the nerve cells.

MATERIALS AND METHODS

Design

A confocal immunofluorescent, cell morphological observation.

Time and setting

This study was conducted at the Department of Histology and Embryology, School of Basic Medical Sciences, Central South University, China, from May 2010 to May 2011.

Materials

All protocols of this study complied with the *Guidance Suggestions for the Care and Use of Laboratory Animals* issued by the Ministry of Science and Technology of the People's Republic of China[26]. Eight healthy adult Sprague-Dawley rats, aged 6–7 months, were used in this study. All experimental animals were provided by the Animal Center, Xiangya School of Medicine, Central South University, China (license No. SCXK (Xiang) 20090004).

Methods

Sample preparation

The animals were deeply anesthetized by intraperitoneal injection of 10% chloral hydrate (4 mg/kg) and perfused through the heart with 0.9% saline solution, followed by 4% paraformaldehyde in 0.1 M phosphate buffered saline (pH 7.4) at 4°C. The brain and the spinal cord were removed and post-fixed in the same fixative at 4°C for 2 hours, then immersed in 15%, 30% gradient sucrose in phosphate buffered saline (pH 7.4) overnight for cryoprotection. Coronal sections (25 μm thick) were prepared with a cryostat (Shandon, England).

Immunofluorescent staining

After pretreatment with 1% bovine serum albumin, sections were incubated overnight at 4°C with chicken anti-Abra antibody (kind gifts from Heart and Lung Research, Bad Nauheim, Germany), followed by incubation with Alexa Fluor 488-conjugated goat anti-chicken IgG (Invitrogen, Carlsbad, CA, USA) for 1 hour at room temperature. The nuclei were stained with DAPI and F-actin was stained with tetraethyl rhodamine isothiocyanate-conjugated Phalloidin (Sigma, St. Louis, MO, USA). To test the specificity of immunostaining, the Abra antibody (kind gifts from Heart and Lung Research) was pre-absorbed with the blocking peptide (kind gifts from Heart and Lung Research) overnight at 4°C in the antibody dilution buffer, and then the neutralized antibody was used for immunostaining as mentioned above. In addition, to test the specificity of the second antibody, negative controls were also performed by omitting the Abra antibody or replacing it with normal chicken IgG. The sections were coverslipped and viewed with a Nikon confocal microscope (Nikon, Japan).

Microscopy and imaging

Images were obtained on a Nikon confocal microscope (Nikon). All published images were processed with a Nikon confocal microscope using the quantitation EZ-C1 3.70 software (Nikon, Japan), rotated and cropped using Photoshop CS5 (Adobe, USA).

Statistical analysis

Three rats were used for statistical analysis of Abra-positive cell counts. Four sets of consecutive 25 μm-thick coronal sections were collected from each brain. One set of sections was processed for the double immunostaining of Abra and F-actin. Immunopositive cells stained for DAPI or Abra were quantified manually under a 40 × objective lens by an observer who was blinded to the condition of the sample. The proportion of the nuclei stained by both DAPI and Abra to the nuclei only stained

by DAPI is considered as the Abra positive neuron index.

Acknowledgments: We thank professor Wolfgang Schaper, Max-Planck-Institute from Heart and Lung Research, Bad Nauheim, Germany for kindly providing chicken anti-Abra antibody, Abra antibody, and blocking peptide.

Funding: This study was partly supported by the National Natural Science Foundation of China, No. 30971532; Ph.D. Programs Foundation of Ministry of Education of China, No. 20090162110063, the Natural Science Foundation of Hunan Province, No. 09JJ5015; the Scientific Research Program of Hunan Provincial Higher Education Institutes, No. 110541.

Author contributions: Lihua Liu and Mingying Luo performed the animal experiments and provided data. Baolin Yang and Xiaoqiong Wu were in charge of data integration and manuscript writing. Wu Zhu and Yinglu Guan were in charge of statistical analysis. Weijun Cai, Kerstin Troidl, Wolfgang Schaper, Jutta Schaper and Lihua Liu were responsible for study design.

Conflicts of interest: None declared.

Ethical approval: This study received permission from Animal Ethics Committee of Central South University in China.

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(Edited by Wang QB, Qiong JB/Song LP)