BRAIN RESEARCH 1367 (2011) 77–84

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Human Ermin (hErmin), a new oligodendrocyte-specific cytoskeletal protein related to epileptic seizure

Tao Wang[a], Lintao Jia[a], Bochang Lv[c], Bei Liu[d], Wei Wang[a], Fang Wang[e], Guodong Yang[a], Xin Bu[a], Libo Yao[a,*], Bin Zhang[a,b,*]

[a]Department of Biochemistry and Molecular Biology, Fourth Military Medical University, Xi'an 710032, China
[b]Department of Hematopoietic Stem Cell Transplantation, Affiliated Hospital to Academy of Military Medical Sciences, No. 8, East Street, Fengtai District, Beijing 100071, China
[c]Department of Anatomy and K.K. Leung Brain Research Centre, The Fourth Military Medical University, Xi'an, China
[d]Department of Neurosurgery and Institute for Functional Brain Disorders, Tangdu Hospital, Fourth Military Medical University, China
[e]Department of Immunology, State Key Laboratory of Tumor Biology, Fourth Military Medical University, Xi'an 710032, China

ARTICLE INFO

Article history:
Accepted 1 October 2010
Available online 8 October 2010

Keywords:
hErmin
Oligodendrocyte
Arborization
Cytoskeleton
Epilepsy

ABSTRACT

During the maturation of oligodendrocytes, cells are characterized by their morphological changes such as the number of process extensions and sheet-like membranes. This process relies critically on cytoskeleton rearrangement, but the molecular mechanisms underlying this are still unclear. Here, we identify human Ermin (hErmin), a novel cytoskeletal molecule that is expressed exclusively in oligodendrocytes in human brain, as a regulator of cytoskeleton rearrangement. *In vitro*, full-length hErmin expression, but not its truncated mutants lacking the actin-binding domain, promote arborization of cultured COS-7 cells and induce marked changes in cell morphology. The most important is that expression of hErmin in specimens of epileptic patients is much lower than that of control, implying that hErmin may be involved in epileptogenic process. These findings suggest a role for hErmin as a novel cytoskeleton-related oligodendroglial protein in human brain myelination and human epileptogenesis, and provide new evidence for the relationship between oligodendrocytes and epilepsy.

1. Introduction

Oligodendrocytes are mainly myelin-forming cells which extend processes to contact and repeatedly wrap around axons (Baumann and Pham-Dinh, 2001). Maturation of oligodendrocytes is necessary for myelination and normal function of the brain (Baumann and Pham-Dinh, 2001; Bradl and Lassmann, 2010). In CNS development, myelin serves as a unique structure which is indispensable for saltatory conduction of nerve impulses (Fields, 2008). Abnormalities or injury in oligodendrocytes usually leads to myelin damage, and always results in diverse mental disorders (Goldschmidt et al., 2009; Mitew et al., 2010).

During oligodendrocyte maturation, cells undergo several stages of morphological changes. Myelin-specific proteins, such as CNPase, proteolipid protein (PLP) and myelin basic protein (MBP) (Fulton et al., 2009), are sequentially expressed in oligodendrocytes and are required for myelination (Kursula,

* *Corresponding authors*. Department of Biochemistry and Molecular Biology, Fourth Military Medical University, 17 Changle West Road, Xi'an, China. Fax: +86 29 84774513.
E-mail addresses: bioyao@fmmu.edu.cn (L. Yao), biozhangb@fmmu.edu.cn (B. Zhang).

0006-8993/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2010.10.003

2008). However, the exact mechanisms underlying the morphological changes during maturation of oligodendrocytes, and during the stages of myelination, are still not clear. Studies have shown that the spatial organization of the cytoskeleton is essential for oligodendrocyte morphogenesis and is critically involved in the formation of myelin (Bauer et al., 2009).

The oligodendrocyte cytoskeleton is characterized by an elaborate network of microtubules (MT) and microfilaments (MF), but is devoid of intermediate filaments (IF) (Bauer et al., 2009; Richter-Landsberg, 2001). As a component of the oligodendrocyte cytoskeleton, MF maintains basic stability of membranes and mediates the outgrowth of primary process (Bauer et al., 2009). Some oligodendrocyte-specific proteins have been identified and characterized for their interaction with MF or MT, causing morphologic changes of oligodendrocytes (Lee et al., 2005; Zhang et al., 2005). In recent years, two oligodendrocyte-specific proteins, Juxtanodin (JN) (Zhang et al., 2005) and Ermin (Brockschnieder et al., 2006), have been identified from rat and mouse brain, respectively. Both of these proteins are components of the myelin sheath and share the same actin-binding domain. It is conceivable that there are more, yet undefined oligodendrocyte-specific molecules that could interact with cytoskeleton elements. The identification and characterization of these molecules would be helpful to study the development of oligodendrocytes and the mechanisms for myelinating and demyelinating diseases.

Epilepsy is one of the major neurological disorders; the population affected by forms of epilepsy is estimated to be approximately 0.8% (Pitkanen and Lukasiuk, 2009). Most epilepsy develops from brain injury whereas in many other patients, the etiopathogenesis for epilepsy is still unknown. During the epileptogenic process, the change most often described is neurodegeneration (Jutila et al., 2002). Studies also have shown that glial cells, such as astrocytes and microglia, can contribute to the epileptogenic process (Hailer, 2008; Wetherington et al., 2008). However, whether oligodendrocyte or myelin pathology is involved in the epileptogenic process is poorly understood. Here we report the identification of a new oligodendrocyte-specific protein (termed "human Ermin") that can induce morphological changes through binding to F-actin. Moreover, we provide evidence that the expression of hErmin is decreased in temporal lobe epilepsy (TLE) patients, suggesting a role for this novel protein and oligodendrocytes in the epileptogenic process.

2. Results

2.1. *hErmin is a novel protein expressed exclusively in human brain*

According to the sequence that is currently annotated in the National Center for Biotechnology Information Entrez Gene database under GeneId 57471 (human KIAA1189), the *hErmin* has two transcript variants that differ in the 5′ UTR and coding region. As a result, isoform b, transcribed from hErmin mRNA variant 2, is shorter at the N-terminus than isoform a, as deduced from the 3.7 kb cDNA clone, with 284 and 297 amino acid residues, respectively. Given that the length of isoform b is closer to Ermin (281 amino acid residues) in mouse (Brockschnieder et al., 2006) and JN (282 amino acid residues) in rat (Zhang et al., 2005), we presumed that hErmin may be expressed or function mainly as isoform b. Using specific PCR primers for isoforms a and b, we successfully cloned an 850 bp fragment from human brain cDNA, and the DNA sequencing results confirmed our presumption. Although the possibility of isoform a expression could not be excluded, the isoform b is likely to be the major transcript of *hErmin*. Therefore, we used isoform b as a target to study the character and function of hErmin.

In comparison with characterized proteins, the C terminus of hErmin showed high homology to the actin-binding domain of the ezrinradixin-moesin (ERM) proteins (Fig. 1A, NCBI Protein Data Bank accession no. for moesin: AAB61666). The boxed residues denote the homologous region among the proteins. However, unlike the ERM proteins, hErmin contains no detectable plasma-membrane-binding FERM (band four-point-one ERM homology) domain.

Western blot analyses of different tissue lysates using an antibody to hErmin detected a 50 kDa single band protein in human brain sample, but not in other tissues (Fig. 1B), suggesting that hErmin is expressed specifically in the CNS and exists as only one isoform, confirming our PCR results.

2.2. *Identification of hErmin in human brain by immunofluorescence*

Immunofluorescence staining of adult brain sections showed that hErmin distributed along myelinated axon/fiber tracts. hErmin co-localized with two myelin-specific protein markers, CNPase and MBP, and intense labeling of hErmin was found along the bodies and processes of oligodendrocytes, indicating that hErmin is an oligodendrocyte-specific protein (Fig. 2A–H). Microscopy at a higher magnification further revealed a more detailed distribution of hErmin. As shown in Fig. 2D and H, hErmin is mainly detected on the boundary of CNPase/MBP positive processes. Most of the hErmin labeling is not continuous but circles around the surface of MBP-labeled internodes. Additionally, hErmin was also detected around or along the neurofilament-200-positive axons (Fig. 2P), implying that the immunostained fibers are the myelinated fibers. No significant hErmin co-localization was found with glial fibrillary acidic protein (GFAP) in astrocytes (Fig. 2I–L), or neurofilament 200 (NF-200) in neurons (Fig. 2M–P). We thus concluded that in adult human brain, hErmin is expressed specifically in oligodendrocytes and may be a component of myelin architecture.

2.3. *Overexpression of hErmin promotes formation of arborized processes in COS-7 cells*

Next we tested whether hErmin could modify cell morphology *in vitro*. In comparison with cells expressing EGFP (Fig. 3A), hErmin-transfected cells exhibited dramatic morphology changes (Fig. 3D). Immunofluorescence staining of these hErmin-expressing cells using hErmin-specific antibody showed intense labeling of fibers in parallel with the membrane in the cytosol (Fig. 3D, E). High expression of hErmin was also detected in short cellular spikes distributed