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PERIPHERAL MYELIN PROTEIN-22 IS EXPRESSED IN CNS MYELIN

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Abstract

Myelin abnormalities exist in schizophrenia leading to the hypothesis that oligodendrocyte dysfunction plays a role in the pathophysiology of the disease. The expression of the mRNA for the peripheral myelin protein-22 (PMP-22) is decreased in schizophrenia and recent genetic evidence suggests a link between PMP-22 and schizophrenia. While PMP-22 mRNA is found in both rodent and human brain it has been generally thought that no protein expression occurs. Here we show that PMP-22 protein is present in myelin isolated from adult mouse and human brain. These results suggest that PMP-22 protein likely plays a role in the maintenance and function of central nervous system (CNS) myelin and provide an explanation for why altered PMP-22 expression may be pathophysiologically relevant in a CNS disorder such as schizophrenia.

Keywords

Myelin; Peripheral myelin protein-22; Schizophrenia

1. Introduction

Multiple lines of evidence including molecular studies, neuroimaging, as well as histopathological and ultrastructural analyses have demonstrated myelin abnormalities in

schizophrenia, leading to the hypothesis that oligodendrocyte dysfunction plays a central role in its pathophysiology (reviewed in [1,2]). In particular, both microarray and quantitative polymerase chain reaction studies showed that expression of a subset of myelin-related genes is decreased in postmortem brain in schizophrenia [3–7]. Peripheral myelin protein-22 (PMP-22) is one of the myelin-related genes whose RNA is decreased in schizophrenia [4,6]. Both a copy number variation in PMP-22 as well as a deletion in chromosome 17p12 which contains the PMP-22 gene have been linked to schizophrenia [8].

PMP-22 is a transmembrane protein whose expression is most abundant in compact myelin of the peripheral nervous system (PNS) [9] where it is important for the establishment and maintenance of myelin [10]. The gene has two alternatively spliced forms containing either exon 1A or 1B, respectively, which are regulated by use of an alternative promoter with form 1A being myelin-specific, whereas form 1B is expressed more broadly [11]. As both exons 1A and B are non-coding, the two forms produce the same protein.

PMP-22 was initially identified as growth arrest specific gene 3 (gas 3) and subsequently as a protein whose expression is severely decreased post-peripheral nerve injury [12]. PMP-22 mRNA is detected in both rodent [9,13] and human brain [14] where relatively high levels of expression are found in the corpus callosum by Northern blotting. However, no protein expression has been detected in Western blots of total brain homogenates [9,13]. Recently it was suggested that the lack of PMP-22 protein expression in the CNS, despite the presence of RNA, results from the fact that in developing oligodendrocytes translation of PMP-22 RNA is negatively regulated by the microRNA mir-9 [15]. Therefore, although present in PNS myelin, PMP-22 is not considered a component of CNS myelin.

Given the apparent lack of PMP-22 protein expression in the CNS, it was surprising that genetic studies identified a link between PMP-22 and schizophrenia [8]. This led us to reexamine whether PMP-22 protein is expressed in the CNS, particularly focusing on white matter. Here we show that PMP-22 protein is present in myelin isolated from adult mouse and human brain.

2. Experimental Procedures

2.1 Isolation of myelin

Myelin was prepared from 3-month old adult mouse brain by density gradient centrifugation [16]. Whole brain or forebrain with brainstem and cerebellum removed were homogenized in 20 mM Tris HCl, pH 7.45, 2 mM EDTA, 1 mM DTT (Tris-HCl buffer), supplemented with Halt protease and phosphatase inhibitor cocktail (Pierce Biotechnology, Rockford IL, USA) and containing 0.3 M sucrose. The homogenate was layered on 0.83 M sucrose in the above buffer and centrifuged at 35,000 g for 35 minutes. The myelin membranes were collected at the interphase of the sucrose solutions and further purified by hypo-osmotic shock in Tris HCl buffer yielding a crude myelin extract. To obtain purified myelin, the sucrose gradient centrifugation and hypo-osmotic shock were repeated once more. For Western blot analysis the myelin fraction was solubilized in 62.5 mM Tris HCl pH 6.8, 10% glycerol, 3% sodium dodecyl sulfate (SDS). As a positive control, mouse sciatic nerves were dissected and extracts prepared as described in Amici *et al.* [17].

For isolation of myelin from human brain, frozen samples dissected from Brodmann area 9 (~1 g each) were obtained from 3 normal cases (ages: 52, 81, 86, years; postmortem interval: average 11.9 hours, range 2.4–21.7 hours) from the Brain Bank of the Department of Psychiatry of the Mount Sinai School of Medicine/James J. Peters VA Medical Center. Myelin was isolated as described above.

2.2 Western blotting

Protein concentrations were determined by the BCA reagent (Pierce) and proteins were separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto polyvinylidene difluoride (PVDF) membranes (Millipore Corporation, Billerica, MA, USA). The membranes were probed overnight at 4°C with either an affinity polyclonal antibody, (Abcam, Cambridge, UK, Ab 61220 diluted 1:750) or a polyclonal anti-PMP-22 antibody raised in chicken (L. Notterpek *et al.* unpublished, diluted 1:1,000). Both antibodies were diluted in TBST (50 mM Tris HCl pH 7.4, 1% Tween) containing 5% low-fat dry milk. After incubation with anti-rabbit horseradish peroxidase (HRP; GE Healthcare Bio-Sciences Corporation, Piscataway, NJ, USA; 1: 5,000 in blocking solution) or anti-chicken IgY-HRP (Jackson Immunolabs, West Grove, PA; 1:100,000) binding of secondary antibodies was visualized with the ECL+ reagent (GE Healthcare Bio-Sciences Corporation). In some experiments myelin extracts were treated for 2 hours at 37°C with PNGase F (New England Biolabs, Ipswich, MA USA) according to the manufacturer's instructions and analyzed by Western blotting.

3. Results and Discussion

Initially, we studied PMP-22 expression in 3-month old mouse brain by Western blotting. As shown in Figure 1A, the PMP-22 protein could not be detected in total brain homogenate even when 100 µg of total protein was analyzed. In comparison, PMP-22 expression was found in the crude myelin fraction isolated from total brain, although its levels were considerably lower than those found in the sciatic nerve (Figure 1A). To substantiate the expression of PMP-22 in CNS white matter, purified myelin fractions were subsequently prepared from total brain and forebrain and analyzed using two different polyclonal antibodies (Figure 1B). As shown on the Western blot, PMP-22 protein expression was detected in myelin isolated from both regions.

We next determined whether PMP-22 could be detected in myelin from human brain. Myelin was isolated from frozen samples dissected from Brodmann area 9 obtained from three subjects. As shown in Figure 2A, in each of the three human brain myelin samples, a band that migrated slightly slower than mouse PMP-22 was detected. This observation is consistent with previous reports indicating that human PMP-22 has a slowed mobility as compared to the mouse protein, probably due to altered post-translational modifications [18]. To further demonstrate that the PMP-22 core protein was present, myelin extracts were treated with PNGase F to remove the N-linked carbohydrate side chain ([19]). Treatment with PNGase F resulted in an approximately 4 kDa shift in the mobility of PMP-22, with a complete disappearance of the 22-kDa band and the appearance of an approximately 18 kDa band (Figure 2B). This pattern was consistent among all four samples analyzed and confirms the expression of the PMP-22 protein in mouse and human white matter.

Although the presence of the PMP-22 mRNA has been documented in both rodent and human brain, detection of the protein has been challenging probably due to its low level of expression. Here we show that PMP-22 protein can be detected by Western blotting in myelin isolated from both mouse and human brain. We have not been able to detect PMP-22 protein by immunohistochemical staining of either frozen sections or perfusion-fixed Vibratome sections from adult mouse brain (data not shown). We suspect that this is likely due to the low level of protein expression and possibly the masking of the antigen in the lipid-rich CNS myelin.

PMP-22 is thought to function as a modulator of cell-cell or cell-matrix interactions that are important for myelin formation and maintenance [10,17,20]. Misexpression of the protein is associated with hereditary demyelinating peripheral neuropathies in humans. Deletion of

PMP-22 is linked with hereditary neuropathy with liability to pressure palsies (HNPP), while duplication of the gene is associated with Charcot-Marie-Tooth disease type 1A [10,12]. CNS white matter lesions have been detected in families with HNPP [21], although the reasons for these findings were unclear. The present demonstration of PMP-22 expression in brain white matter suggests that this protein may play a role in the proper maintenance and function of CNS myelin and points to a need for further studies addressing its role in normal CNS function and disease. Our data also provide an explanation for why changes in PMP-22 expression may be relevant to the pathophysiology of schizophrenia [4,6,8].

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Figure 1.

Detection of PMP-22 protein in CNS myelin by Western blotting. (A) Crude myelin fraction was isolated from total brain of a 3-month old mouse by density gradient centrifugation and myelin proteins were analyzed by Western blot with a rabbit polyclonal anti-PMP-22 antibody. Gels were loaded with 100 μ g of protein from a non-fractionated total brain homogenate (Br), 25 μ g from an isolated myelin preparation or as a positive control with increasing amounts (1, 3, or 5 μ g) of sciatic nerve homogenates (SN). (B) Purified myelin was isolated from total brain and forebrain of a 3-month old mouse and analyzed for PMP-22 expression as above. The lane indicated Mu(FBr) contains 25–30 μ g of protein from a forebrain purified myelin preparation; Mu(TB) contains 25–30 μ g of a total brain myelin preparation and SN was loaded with 100 ng of a mouse sciatic nerve homogenate. The upper panel was probed with the rabbit polyclonal anti-PMP-22 antibody used in (A), while the lower panel was probed with a chicken polyclonal anti-PMP-22 antibody. Molecular masses are indicated on the left.

Figure 2.

(A) Myelin was isolated from frozen human brain samples from three different subjects and analyzed for PMP-22 expression. Gels were loaded with 25–30 μ g of protein from human or mouse brain (MuBr) myelin fractions or with 50 ng of a mouse sciatic nerve homogenate (SN). (B) Treatment of brain protein samples with PNGase F. Myelin fractions, 30–50 μ g of protein from human brain (HuBr), total mouse brain (MuBr), mouse forebrain (MuFBr) or 50 ng of sciatic nerve extract (SN) were digested with PNGase F for 2 hours at 37°C and reacted with anti-PMP-22 antibody. Note that upon treatment with PNGase F, the ~22 kDa band is converted to an ~18 kDa band, the size of the PMP-22 core peptide.