Amyloid precursor protein at node of Ranvier modulates nodal formation

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Amyloid precursor protein (APP), commonly associated with Alzheimer disease, is upregulated and distributes evenly along the injured axons, and therefore, also known as a marker of demyelinating axonal injury and axonal degeneration. However, the physiological distribution and function of APP along myelinated axons was unknown. We report that APP aggregates at nodes of Ranvier (NOR) in the myelinated central nervous system (CNS) axons but not in the peripheral nervous system (PNS). At CNS NORs, APP expression co-localizes with tenascin-R and is flanked by juxtaparanodal potassium channel expression demonstrating that APP localized to NOR. In APP-knockout (KO) mice, nodal length is significantly increased, while sodium channels are still clustered at NORs. Moreover, APP KO and APP-overexpressing transgenic (APP TG) mice exhibited a decreased and an increased thickness of myelin in spinal cords, respectively, although the changes are limited in comparison to their littermate WT mice. The thickness of myelin in APP KO sciatic nerve also increased in comparison to that in WT mice. Our observations indicate that APP acts as a novel component at CNS NORs, modulating nodal formation and has minor effects in promoting myelination.

Introduction

Amyloid precursor protein (APP) is best known for its involvement in the pathogenesis of Alzheimer disease (AD). Increasing evidence suggests that damage to myelin may be an important component of AD.1-3 It has been suggested the measures of white matter tract integrity may be a viable neuroimaging biomarkers of the earliest stages of Alzheimer disease.4 In a mouse transgenic model of Alzheimer disease expressing mutant APP and presenilin-1, extensive myelin pathology and disruption of myelinated fibers was observed in association with amyloid plaques.5 Moreover, APP is upregulated and distributes evenly along the injured axons, and therefore, also known as a marker of demyelinating axonal injury and axonal degeneration. APP expression is upregulated in axons on injury and has long been utilized as a marker for axonal degeneration.6,7 Demyelinating injury, such as in multiple sclerosis, is also associated with increased axonal APP expression.8,9 However, the exact distribution and function of APP along myelinated axons has yet to be elucidated. An understanding of how APP is associated with myelinated axons is important, as myelination is a vital biological process disrupted in a wide variety of congenital and acquired neurological diseases.

Myelinated axon is characterized by their segmental structures: axonal initial segment (AIS), node of Ranvier (NOR), paranode, juxtaparanode, and internode.10 The establishment and maintenance of the elaborate architecture of axonal domains and the congregation of specific ion channels and recognition molecules, such as Nav1.6 sodium channels, F3/contactin, tenascin-R, and Oligodendrocyte myelin glycoprotein (OMgp), at NORs are critical to ensuring rapid saltatory conduction of action potentials along myelinated fibers.11 In the present study, we have shown that APP localizes at NORs in the central nervous system (CNS) and modulates nodal formation.
Results

APP clusters at NORs in the CNS, but not PNS

To investigate APP distribution along myelinated axons in the CNS, the longitudinal sections of rat spinal cord were stained for APP and Kvβ2 (Potassium channel β2 subunit; a marker to label juxtaparanode12) (Fig. 1A) or Tenascin-R (TnR; a marker to label NOR13) (Fig. 1B) by immunofluorescence (IF). APP immunoreactivity was evenly distributed along myelinated fibers in the adult rat sciatic nerve. This was shown by double immunofluorescence labeling with APP and juxtaparanodal potassium channels Kv1.2 (Fig. 1D). The specificity of APP antibody was tested previously and confirmed by lack of immunoreactivity in APP KO mice.15-18 These observations demonstrate that axonal APP is a novel component of the NORs and its clustering occurs specifically in the CNS, but not in the PNS.

Myelination is a dynamic process mediated by bidirectional axoglial interactions that are involved in the clustering of axonal molecules, such as sodium channels and F3/contactin, at the NORs.19 Next, we studied the aggregation of APP in correlation with sodium and potassium channel clustering during nodal maturation. At P6, before sodium channels start clustering at the NORs, APP was diffusely distributed along axons (Fig. 2A). APP appeared to start clustering in the NORs around P10 (Fig. 2A) and was prevalent after P15 when the nodal structure has been formed (Fig. 2A). These observations indicate that APP clustering lags behind sodium channel clustering in the CNS. The intensity of APP nodal clustering was quantified in relation to Kv1.2 clustering during development. The ratio of APP clustering to Kv1.2 clustering, reflecting the developmental progress of APP clustering in the population of axons, was around 50% at P10, 60% at P15 and P21, and 90% at 2 mo (Fig. 2B). These results support the notion that nodal accumulation of APP requires the integrity of distinct axonal domains, as is the case for other compartmentalized axonal proteins.43

APP deficiency increases the length of NORs

Structural maturation of NORs is essential for salutatory conduction, which is completed during the late phase of myelination.21-23 To define whether APP may play a regulatory role in nodal formation, we analyzed nodal structures in the spinal cord of APP KO as well as their WT mice. As reflected by double-staining using antibodies against Nav1.6 and Caspr (Contatin associated protein; a paranode marker), nodal length increased in axons of the spinal cord of APP KO vs. WT mice (Fig. 3A). Meanwhile, neither Nav1.6 (Fig. 3A) nor Tenascin-R (not shown) clustering showed any discernible alternation in the APP KO vs. their WT littermates. Quantification showed that nodal length was significantly increased in the APP KO vs. WT mice (Fig. 3B).

Moreover, we analyzed nodal structures in the spinal cord of APP KO mice as well as their WT mice using electronmicroscopic (EM) approach. Ultra-structural observations revealed that the lateral oligodendrocytic loops remained compacted in APP KO mice, as they are in WT mice (Fig. 3C). Ultra-structural quantification showed that the nodal length was significantly reduced in the APP KO mice vs. WT littermates (Fig. 3D). Together, these observations suggest that APP plays a role in modulating the length of NORs.

APP has minor effect on myelination

Nodal molecules, which usually interact with glial molecules, taken an instance, Omgp, modulate myelination through axon-glia interaction.24 Considering that APP localizes at NORs, we therefore investigated whether APP might modulate myelination using electronmicroscope (EM) analysis. The degree of myelination in spinal cord from 3-mo-old APP WT and KO mice as well as transgenic (APP TG) mice which overexpress human APP driven by the prion promoter25 was analyzed. EM showed no axonal swelling in KO and transgenic mice at this age (Fig. 4A, D, and G). Interestingly, the g ratio, the numeric ratio between the diameter of the axon and the outer diameter of the myelinated fiber,26 which affects the thickness of myelin (Smaller g ratio indicates thicker myelin), was increased in the spinal cord of APP KO mice (0.829 ± 0.002) in comparison to WT mice (0.817 ± 0.002) (Fig. 4B). Although statistic significances in the g ratio were observed, the g ratio in APP KO spinal cord showed only 2% increase compared with that in APP WT mice. To confirm these results, we analyzed the spinal cord from 3-mo-old APP TG mice. As predicted, the g ratio was decreased in APP TG spinal cord compared with WT spinal cord (Fig. 4E). The g ratio in APP TG spinal cord showed a 1.3% decrease compared with that in the littermate WT mice. However, the axonal distribution showed no differences in between the spinal cord of APP KO, APP TG, and their littermates WT mice (Fig. S1A and B). Thus, APP only has a minor effect on promoting myelination in the spinal cord. Moreover, the average g ratio in APP KO sciatic nerve increased (Fig. 4H), and showed a 5% increase in comparison to that in APP WT sciatic nerve, while the axonal distribution in APP KO sciatic nerve showed no differences from WT sciatic nerve (Fig. S1C). These results indicate that APP promotes myelination in sciatic nerve.

Discussion

We have found that APP is clustered at the NORs of myelinated axons in the CNS but not in the PNS. APP, as a novel component at CNS NORs, is involved in negatively modulating nodal length in the CNS.

During myelination, various axonal and glial molecules redistribute themselves to take up defined domains through dynamic axoglial interaction.10 NORs are the naked regions of the axolemma and intermittently distribute along myelinated axons,
Figure 1. APP localizes at nodes of Ranvier in the CNS, but not in the PNS. (A and B) The longitudinal sections of spinal cord of 2-mo-old rat were stained for APP (A and B) and Kvβ2 (A) or tenascin-R (TnR; B). The Z-stack confocal images were showed in the right panels. Scale bars: 10 μm. (C) The longitudinal sections of optic nerves and cerebellum of 2-mo-old rat were stained for APP and tenascin-R (TnR). Scale bar: 20 μm. (D) The teased sciatic nerve were stained for APP and neurofilament200 (NF200) or Kv1.2. Scale bars: 10 μm.
implying potential interactions between exposed axonal molecules and the extracellular partners. Over the past decades, the molecular composition corresponding to the morphological domains of the myelinated axon has been extensively studied, with a major focus on the cell adhesion molecules. For instance, F3/contactin, a member of the immunoglobulin superfamily, has been implicated in molecular communication in both nodal and paranodal sites of the CNS, via interacting with sodium channels and Caspr/paranodin, respectively. TAG-1/TAX, from the same L1 cell adhesion molecule subfamily as F3/contactin, has been suggested to be a constituent of juxtaparanodal formation. The sodium channel β subunits, which associate with α subunits of voltage-gated sodium channels at the NORs, have an extracellular immunoglobulin-like loop and act as cell adhesion molecules. APP also plays a role in cell adhesion and interacts with various cell adhesion molecules, including TAG-1/TAX. Previously, it has been noted that APP expression in axons is upregulated on neural injury, and hence, APP expression has been utilized as a marker for axonal degeneration. Moreover, following demyelinating injury, APP expression was also increased. We found that in the absence of injury, APP was largely aggregated at NORs along axons in the CNS. The diffusely distribution of APP along the injured axons may be due to the fact that the clustering of nodal proteins such as sodium and potassium channels requires the integrity of myelinated axons.

We further investigated the pattern of APP clustering at NORs during development. APP clustering occurred later than the clustering of both sodium and potassium channels. Consistent with this fact, APP is not required for clustering of other nodal molecules such as tenascin-R, Caspr, and sodium channels, as clustering

Figure 2. Location of APP in the spinal cord during development. (A) The longitudinal sections of spinal cord of rats at postnatal day 6 (P6), P10, P15, and P21 were stained for APP and sodium channel (Pan; P6) or Kv1.2 (Kch; P10, 15, and P21). Scale bars: 10 μm. (B) The numbers of APP+ nodes were quantified and expressed as the percentage of APP+ nodes and Kch+–defined gaps. Data are presented as mean ± SEM.
of these molecules still occurs in APP KO mice. Thus, it is clear that APP is not an initiator for the formation of NORs. However, the nodal gap is abnormally widened in APP KO mice, indicating that APP may function in generating normal nodal architecture and in maintaining the physiological functions of NORs. Various proteins have been identified to maintain the normal architecture and functions of NORs, which includes axonal and glial molecules. It is supposed that the axon–glial interaction play essential roles in nodal formation. APP is expressed in both axons and glial cells, including astrocytes and oligodendrocytes. The nodal APP we have observed in this study was detected by an antibody recognizing the intracellular domain of APP. Therefore, it is unlikely that the nodal APP is secreted from glial cells, like TnR. However, how axonal APP modulates nodal length remains unclear. One possibility is that APP interacts with other nodal proteins such as ankyrin G, Neurofascin 155, and TnR, modulating the formation of NORs. APP also possibly interacts with paranodal protein, through which, anchoring the paranodal loops to the boundary of NORs. Identification of the binding partners of APP at NORs will help to address such possibilities.

Myelination is a multi-step coordinated process involving cellular interactions between axons and oligodendrocytes in the CNS and Schwann cells in the PNS. Interestingly, reduced myelination thickness, as reflected by increased g ratios, occurs in both CNS and PNS of APP KO mice, implying that APP promotes myelination in both CNS and PNS. However, the alterations of g ratio in both APP KO and APP TG spinal cords are about 2% in comparison to that in their WT littermates. The g ratio in the sciatic nerve of APP KO mice has 5% increase compared with that in WT mice. These results indicate that APP only has minor effects on myelination. Considering the different location of APP in the PNS and CNS, it is unlikely that the effects of APP on myelination in both the PNS and CNS are related to the nodal location of APP.

APP plays essential roles in Alzheimer disease as it generates amyloid-β by proteolytic cleavage. Impaired myelinated axons have been detected in the brains of Alzheimer disease patients and been supposed as an early component of Alzheimer disease. Abnormalities in CNS myelination affect the molecular organization at and around nodal axons leading to disturbances in CNS functions. For example, the heterozygous proteolipid protein transgenic mice exhibited profound reduction in Nav1.6 clusters, loss of the paranodal axo-glial apparatus, and a marked increase in Nav1.2. Dysfunction of nodes of Ranvier are now recognized as key contributors to the pathophysiology of various neurological diseases. It requires more investigation on the functions of the nodal APP, especially how the nodal APP contributes to Alzheimer disease.

Figure 3. Increased nodal length in APP KO spinal cord. (A) The longitudinal sections of spinal cord from APP-KO and WT mice at 2 mo old were stained for Caspr and sodium channels (Nach). Scale bars: 10 μm. Scale bars in higher magnification: 5 μm. (B) Nodal lengths in APP KO vs. WT mice were analyzed. The nodal length in APP WT spinal cord was normalized to 1.0. The relative nodal length in APP KO spinal cord was quantified and compared. Data are presented as mean ± SEM. *P < 0.05. (C and D) EM analyses of longitudinal spinal cord sections of APP KO vs. WT mice (all aged 3 mo). The red dotted lines and bars mark the spans of nodal gaps. Scale bars: 500 nm. The nodal length in APP WT spinal cord was normalized to 100. The relative nodal length in APP KO spinal cord was quantified and compared. Data are presented as mean ± SEM. *P < 0.05.
Materials and Methods

Antibodies, peptides, inhibitors, and activators

Rabbit polyclonal antibodies against Caspr43 and monoclonal antibodies against Tenascin-R (596, 619) were generously from Dr. Dennis Selkoe (Harvard University; C7 and C9). Monoclonal antibodies against pan-sodium channel (Sigma), potassium channels Kv1.2 (K14/16), Kvβ2 (Upstate), and NF200 (Sigma) were obtained from the respective commercial sources.

Mutant mice

The APP KO and APP overexpressing TG mice were as previously described. All experiments involving mice were approved by the Institutional Animal Care and Use Committee of Soochow University.

Immunofluorescence labeling

Animals were perfused sequentially with 0.1 M Ringer’s solution and 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB). Spinal cords were removed immediately. For cryosectioning, tissues were immersed in 0.1 M phosphate buffered saline (PBS) at 4 °C for later use. Sciatic nerve was teased as previously described.

| Figure 4. | The effects of APP on myelination. (A) EM studies of cross-sections revealed the ultrastructure of myelin sheaths in spinal cords (SC) of WT vs. APP KO mice (all aged 3 mo). Scale bars: 2 μm. (B) The g ratio in APP WT and KO spinal cord was analyzed. Data are presented as mean ± SEM. **P < 0.01. (C) The g ratio in APP WT spinal cord was normalized as 100%. The relative g ratio in APP KO spinal cord was shown. (D) EM studies of cross-sections revealed the ultrastructure of myelin sheaths in the spinal cord (SC) of WT littermates vs. APP TG mice (all aged 3 mo). Scale bars: 2 μm. (E) The g ratio in APP WT and TG spinal cord was analyzed. Data are presented as mean ± SEM. **P < 0.01. (F) The g ratio in APP WT spinal cord was normalized as 100%. The relative g ratio in APP TG spinal cord was shown. (G) EM studies of cross-sections revealed the ultrastructure of myelin sheaths in sciatic nerves (SN) of APP WT and KO mice (all aged 3 mo). Scale bars: 5 μm. (H) The g ratio in the sciatic nerve of APP WT and KO at 3 mo old was analyzed. Data are presented as mean ± SEM. **P < 0.01. (I) The g ratio in APP WT sciatic nerve was normalized as 100%. The relative g ratio in APP KO sciatic nerve was shown. |
acetone. After undergoing gradual infiltration with Araldite 502, the tissue was dehydrated in an ascending series of alcohol and ethyl alcohol, and thereafter, subjected to dehydration in an ascending series of alcohol and acetone. After undergoing gradual infiltration with Araldite 502 (EMS), the blocks were embedded and polymerized overnight at 60°C. Ultrathin sections (~90 nm in thickness) were placed on 200-mesh copper grids and counterstained with uranyl acetate and lead citrate. All samples were examined and photographed under a Jeol 1220 electron microscope.

Quantification

The numbers of APP+ NORs, the nodal length was counted from more than 3 different sections each mouse. More than 3 mice were analyzed each group. The length of node in the IF and EM analysis was counted using Image J software. The diameters of axons and fibers in more than 10 pictures taken under 6000x magnification from each mouse were counted using Image J software. At least 3 mice were analyzed in each group. The g ratios were calculated as axonal diameter/fiber diameter.

Data analysis

All values were expressed as mean ± SEM, where n indicated the number of mice. Student t test was performed to determine whether there were significant differences followed by Bonferroni test as post hoc analysis; *P < 0.05; **P < 0.01.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental material may be found here: www.landesbioscience.com/journals/celladhesion/article/28802.

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