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Differential modulation of the juxtaparanodal complex in Multiple Sclerosis

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ABSTRACT

Myelinated fibers are divided into discrete subdomains around the Na_v-enriched nodes of Ranvier: the paranodes, where axoglial interactions occur, the juxtaparanodes, where voltage-gated potassium channels (VGKCs) are aggregated, and the internode. Perinodal changes have been reported in Multiple Sclerosis (MS) with functional consequences for the axon. Here we report on alterations of the juxtaparanodal proteins TAG-1, Caspr2 and VGKCs in normal appearing white matter (NAWM), perilesion and chronic lesion areas in post-mortem white matter tissue from MS patients compared to control white matter. We show that the molecular organization and maintenance of juxtaparanodes is affected in lesions, perilesions and NAWM in chronic MS through protein and mRNA expression as well as immunohistochemistry. The three molecules analyzed were differentially altered. TAG-1 clustering at juxtaparanodes was reduced in NAWM; TAG-1 and Caspr2 are diffused in perilesions and absent in lesion areas. VGKCs were no longer enriched at juxtaparanodes either at the NAWM or the perilesion and demyelinated plaques. While the protein levels of the three molecules showed only a tendency of reduction in the plaques, there was a significant upregulation of Caspr2 mRNA in the lesions accompanied by a transcriptional increase of paranodal Caspr, indicating an axonal homeostatic mechanism.

1. Introduction

Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by foci of inflammation accompanied by demyelination, axonal loss and glial scar formation (Lassmann, 1998; Prineas et al., 2001). Although the primary etiology of the disease is unknown, the neurological decline that is observed is attributed to the neuronal and axonal loss caused by chronic local inflammation and generalized immune system activation in the CNS (Frischer et al., 2009; Lassmann et al., 2012; Weiner, 2009). This view is supported by the fact that patients suffering chronically from MS are refractory to treatments that suppress the peripheral immune system (Compston and Coles, 2008; Zamvil and Steinman, 2003). In addition to the demyelinating plaques that are seen both in the white and gray matter, the normal appearing white matter (NAWM) has recently been recognized as an area of diffuse pathology (Ciccarelli et al., 2001; Filippi et al., 1999). Among the pathological hallmarks observed are the presence of activated microglia, neurodegeneration, a compromised blood–brain barrier (BBB) and increased glutamate concentration (Howell et al., 2010; Kutzelnigg et al., 2005; Plumb et al., 2002; Srinivasan et al., 2005; Tisell et al., 2013).

Under normal conditions in the adult CNS, a myelinated fiber is characterized by the presence of specialized areas at periodic intervals, the so-called nodes of Ranvier. These sites are enriched in voltage-gated sodium channels (Na_v), which are responsible for the saltatory conduction of the action potential. Next to the nodes are the paranodes (PNs), where the glial and axonal membranes are in close proximity. The PNs constitute a physical and molecular barrier against the diffusion of the nodal components, through the interactions between two axonal surface proteins, contactin-associated protein-1 (Caspr) and Contactin-1 (Cntn-1) as well as the 155-kDa isoform of Neurofascin (Nfasc155) on the glial surface (Charles et al., 2002; Labasque and Faivre-Sarrailh, 2010; Menegoz et al., 1997; Peles et al., 1997; Rios et al., 2000; Sherman et al., 2005; Susuki and Rasband, 2008). In close proximity to the PNs

Abbreviations: VGKCs, voltage-gated potassium channels; MS, Multiple Sclerosis; NAWM, normal appearing white matter; CNS, central nervous system; BBB, blood–brain barrier; Na_v, voltage-gated sodium channel; PN, paranode; JXP, juxtaparanode; Caspr, contactin-associated protein-1; Cntn-1, Contactin-1; Nfasc155, Neurofascin 155; TAG-1, Transient Axonal Glycoprotein-1; Caspr2, contactin-associated protein-1; EAE, experimental autoimmune encephalomyelitis; CFA, Complete Freund's Adjuvant; WM, white matter; IBA1, ionized calcium-binding adapter molecule 1; MOG, myelin oligodendrocyte glycoprotein; MBP, myelin basic protein; CAL, chronic active lesion; CIL, chronic inactive lesion; IHC, immunohistochemistry.

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another specialized area is formed, the juxtaparanode (JXP), characterized by the clustering of the voltage-gated potassium channels (VGKCs) due to their interaction with the scaffold molecules Transient Axonal Glycoprotein-1/Contactin-2 (TAG-1/Cntn-2, from now on referred to as TAG-1) and contactin-associated protein-2 (Caspr2) (Labasque and Faivre-Sarrailh, 2010; Poliak and Peles, 2003; Poliak et al., 2003; Savvaki et al., 2010; Traka et al., 2002, 2003). Proper formation and maintenance of each of these domains are essential for normal neuronal excitability and signal transduction, as well as for neuronal homeostasis.

Disorganization of nodal, paranodal and juxtaparanodal components has been reported in MS lesions. Specifically, in early active demyelinating MS lesions, the paranodal molecules Nfasc155 and Caspr are found in elongated clusters, while VGKCs overlap with the PNs due to their diffusion towards nodal areas (Coman et al., 2006; Howell et al., 2006). As demyelination proceeds, paranodal clusters are even more increased in length and VGKCs diffuse further. In chronic inactive lesions (CILs), paranodal Caspr is completely absent in the lesions and diffuse in the perilesions, where juxtaparanodal Caspr2 and VGKC appear diffused, as well. Na_v clusters are also increased in length in the plaques, meaning that they are the last component to get disorganized (Coman et al., 2006). However, in partially remyelinated plaques, intermediate-sized Na_v+ aggregates, Nfasc155 + and Caspr + PNs as well as Caspr2 + and VGKC + JXPs appear, while their length is restored to normal levels in fully remyelinated tissue (or shadow plaques) (Coman et al., 2006; Howell et al., 2006). We have recently shown in two different rodent models that during demyelination, initially the PNs elongate with subsequent JXP diffusion and downregulation at the protein level. During remyelination, however, the PNs were found to form again followed by JXPs at later stages (Zoupi et al., 2013). Additionally, an activated microglia-mediated effect of paranodal disruption has been described not only in MS NAWM, but also in presymptomatic experimental autoimmune encephalomyelitis (EAE) and CFA (Complete Freund's Adjuvant) injected mice (Howell et al., 2010; Zoupi et al., 2013).

Among juxtaparanodal molecules TAG-1, a GPI-anchored protein of the immunoglobulin superfamily, is of particular interest. It has been identified in a screening for possible autoantigens in MS and is one of the two molecules that are known to mediate axonal pathology under autoinflammatory conditions, with the second one being Nfasc186, as shown in a rodent EAE model (Derfuss et al., 2009; Lindner et al., 2013). Moreover, it is the only perinodal molecule that is expressed by both the neuronal and glial membranes. *Tag-1*$^{-/-}$ mice show hypomyelination in the optic nerve and selective loss of small caliber axons, loss of Caspr2 and VGKC diffusion towards the internode, as well as behavioral deficits (Chatzopoulou et al., 2008; Savvaki et al., 2008, 2010; Traka et al., 2003). Furthermore, the glial isoform alone is sufficient for rescuing the mutant phenotype, pointing clearly to the importance of this molecule for the juxtaparanodal molecular architecture (Savvaki et al., 2010).

In this study we focused on the expression and localization of TAG-1 in relation to its juxtaparanodal partners, Caspr2 and VGKCs, as well as paranodal Caspr, in post-mortem tissue from MS patients. We performed an extensive analysis of NAWM, perilesion areas and chronic plaques (active and inactive) derived from post-mortem MS brain in relation to control white matter (WM), using immunohistochemical, immunoblot, and mRNA analysis for all three juxtaparanodal proteins. We focused on the study of perinodal molecules and markers of demyelination and axonal pathology. Our results demonstrate that JXPs are disrupted not only in lesions, but also in perilesion and NAWM areas in chronic MS. VGKCs were the most susceptible to disruption, followed by TAG-1 and Caspr2. Changes were also detected in the expression levels of all three proteins. Our study is the first to comparatively analyze the localization and expression of all three components of the juxtaparanodal complex in chronic MS tissue. Additionally, a phenotype of juxtaparanodal loss is described for the first time in MS NAWM.

2. Materials and methods

2.1. Human brain tissue samples

Post-mortem human brain samples were obtained from the UK MS Society Tissue Bank (http://www.ukmstissuebank.imperial.ac.uk/). All tissues have been collected following fully informed consent by the donors via a prospective donor scheme according to Ethics Committee approval. Neuropathological analysis was performed on 10 μm-thick cryosections obtained from brain tissue blocks of 1 cm^3 from 10 MS and 9 age-matched controls (see Table 1) that had been frozen either unfixed or fixed (for a minimum of 4 h in 4% paraformaldehyde (PFA)), cryoprotected in 30% sucrose in 1× Phosphate Buffered Saline (1×PBS) and kept at −80 °C. All tissue blocks were processed using immunohistochemistry against ionized calcium-binding adapter molecule 1 (IBA1) and myelin oligodendrocyte glycoprotein (MOG) to assess inflammation and demyelination.

2.2. Characterization and classification of MS lesions

MS lesions were classified according to published criteria (Frohman et al., 2006; Lassmann et al., 1998; Lucchinetti et al., 1996) and most recently published criteria from the UK MS Society Tissue Bank (Reynolds et al., 2011). Chronic active slowly expanding lesions (referred to as chronic active lesions—CALs) were characterized by mild to moderate microglia activation and macrophage infiltration at the lesion margins, and early myelin degradation products restricted to single macrophages (Prineas et al., 2001). Chronic inactive lesions (CILs) were those that had a sharp lesion edge lacking macrophage or microglia infiltration. Areas of NAWM were considered at least at 1 cm away from demyelinated plaques and excluding fiber tracts emerging from white matter lesions. Information on neuropathology of all cases and related details are included in Table 1.

2.3. RNA extraction, reverse transcription and real-time PCR

Extraction of total RNA was performed on samples from control WM (n = 6 cases) and MS NAWM (n = 5 cases, 1 primary progressive (PPMS) and 4 secondary progressive MS (SPMS) cases), chronic active (n = 5 lesions from 4 cases, 1 from a PPMS case and 4 from SPMS cases) and chronic inactive (n = 4 lesions from 3 cases, 2 lesions from a PPMS case and 2 lesions from two SPMS cases) lesions (see Table 1) in immunohistochemically characterized snap-frozen human brain tissue blocks (Markoullis et al., 2012) using the RNeasy Lipid Tissue Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Total RNA was subjected to reverse transcription using the TaqMan RT-PCR reagents and a GeneAmp PCR System (Applied Biosystems, Life Technologies, Thermo Fisher Scientific Inc., Waltham, MA). Expression levels of genes encoding TAG-1 (*Cntn2*, forward primer sequence: CAGTGGGCACCGTTGTCA, reverse primer sequence: TTGAGG AGCCAGCGGTAG), CASPR2 (*Cntnap2*, forward primer sequence: GAAG GTCGCATTGGACTCA, reverse primer sequence: TGCTGTCCTTCTCCGT GC), CASPR (*Cntnap1*, forward primer sequence: CTTGTATTTAGGGCGT GTGA, reverse primer sequence: CGCAATTAGATTCGGACAGT) and GAPDH (*Gapdh*, forward primer sequence: GAAGGTGAAGGTCGGA GTC, reverse primer sequence: GAAGATGGTGATGGGATTTC) were examined by quantitative real-time PCR analysis using a StepOnePlus real-time PCR system (Applied Biosystems, Life Technologies, Thermo Fisher Scientific Inc., Waltham, MA). *Gapdh* was used as the 'housekeeping' control gene. PCR runs were performed for each sample in duplicates using cDNA corresponding to 10 ng of total RNA. Expression levels for each gene in MS samples (NAWM and chronic lesions) were calculated as the fold induction value (2^{DDCt}) relative to control cases and to MS NAWM (only chronic lesions). Data are expressed as mean ± standard error of the mean and statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad