Review **Open Access**

Christiane Richter-Landsberg*

Protein aggregate formation in oligodendrocytes: tau and the cytoskeleton at the intersection of neuroprotection and neurodegeneration

DOI 10.1515/hsz-2015-0157
Received April 10, 2015; accepted June 11, 2015; previously published online June 17, 2015

Abstract: Oligodendrocytes are dependent on an intact, dynamic microtubule (MT) network, which participates in the elaboration and stabilization of myelin forming extensions, and is essential for cellular sorting processes. The microtubule-associated protein tau is constituent of oligodendrocytes. During culture maturation it is developmentally regulated and important for MT stability, MT formation and intracellular trafficking. Downregulation of tau impairs process outgrowth and the transport of myelin basic protein (MBP) mRNA to the cell periphery. Cells fail to differentiate into MBP-expressing, sheet-forming oligodendrocytes. Tau-positive inclusions originating in oligodendrocytes and white matter pathology are prominent in frontotemporal dementias, such as Pick's disease, progressive supranuclear palsy and corticobasal degeneration. An impairment or overload of the proteolytic degradation systems, i.e. the ubiquitin proteasomal system and the lysosomal degradation pathway, has been connected to the formation of protein aggregates. Large protein aggregates are excluded from the proteasome and degraded by autophagy, which is a highly selective process and requires receptor proteins for ubiquitinated proteins, including histone deacetylase 6 (HDAC6). HDAC6 is present in oligodendrocytes, and α-tubulin and tau are substrates of HDAC6. In this review our current knowledge of the role of tau and protein aggregate formation in oligodendrocyte cell culture systems is summarized.

***Corresponding author: Christiane Richter-Landsberg,** Department of Neuroscience, Molecular Neurobiology, University of Oldenburg, P.O. Box 2503, D-26111 Oldenburg, Germany; and Research Center Neurosensory Science, University of Oldenburg, D-26111 Oldenburg, Germany,
e-mail: Christiane.Richter.Landsberg@Uni-Oldenburg.de

Keywords: autophagy; HDAC6; microtubules; posttranslational modification; protein acetylation.

Introduction

The failure to clear misfolded or aggregated proteins is a characteristic feature of a number of neurodegenerative disorders termed proteinopathies or aggregopathies (Dohm et al., 2008; Jellinger, 2012). Proteinaceous inclusions occur in nerve cells and glia, and their composition and appearance is characteristic for each type of disease and used as a diagnostic hallmark. The microtubule-associated protein (MAP) tau is the major constituent of pathological inclusions in Alzheimer's disease (AD) and in frontotemporal disorders such as progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and Pick's disease (PiD), which are classified as tauopathies. While in AD deposits of tau are mainly prominent in nerve cells, in PSP, CBD and PiD tau inclusions occur in neurons and glia, and specifically originate in oligodendrocytes, the myelin forming cells of the central nervous system (Miller et al., 2004; Ballatore et al., 2007; Richter-Landsberg, 2008). These tau-positive inclusions, termed coiled bodies, vary in size and number. Besides tau the inclusion bodies often contain other cytoskeletal proteins and positively stain with antibodies against a variety of heat shock proteins and ubiquitin (Chin and Goldman, 1996; Richter-Landsberg and Bauer 2004; Richter-Landsberg and Goldbaum, 2007). Furthermore, tau-positive globular oligodendroglial inclusions have been identified in motor neuron diseases and frontotemporal dementias, termed globular glial tauopathies (Ahmed et al., 2013).

Tau is a microtubule binding protein promoting microtubule (MT) assembly and stability. Its MT-binding activity is regulated by posttranslational modifications, mainly by phosphorylation, but also glycation, ubiquitylation, sumoylation and acetylation have been reported

(Himmelstein et al., 2012). Tau can be phosphorylated by many kinases and becomes hyperphosphorylated in AD and other tauopathies. This has an influence on its pathological state and may promote a dissociation from the MTs, aggregation and a gain of toxic functions (Gendron and Petrucelli, 2009). In the healthy human brain six tau isoforms are expressed with molecular weights in the range of 45–65 kDa. These derive from a single gene (*MAPT*) by alternative splicing, three comprise of four MT-binding repeats (4R-tau) and three have three MT-binding repeats (3R-tau) (Figure 1). The ratio of 4R- to 3R-tau is approximately 1:1, which may be altered in pathological lesions. Also, mutations in the tau gene may alter the relative proportions of the tau isoforms (Ballatore et al., 2007; Gendron and Petrucelli, 2009). In AD all six isoforms are present in the aggregates, whereas PSP and CBD are considered 4R-tauopathies. In PiD, which is a 3R-tauopathy, Pick bodies containing 3R-isoforms are characteristic (Jellinger, 2009). Hence a disbalance of the 3R/4R-ratio contributes to neurodegeneration.

Furthermore, tau has many interaction partners besides microtubules and is involved in the regulation of signaling cascades (Morris et al., 2011). For example it can bind to SH3 domains of other proteins such as the tyrosine kinase Fyn (Lee et al., 1998). The interaction of tau and α-tubulin with active Fyn has been demonstrated to recruit MTs to the plasma membrane and may be involved in the organization of process formation in oligodendrocytes (Klein et al., 2002).

For a long time tau was considered as a neuron specific protein, so its presence in inclusion bodies and its origin in oligodendrocytes was an enigma and hard to explain until it was demonstrated to also be an oligodendroglial constituent (LoPresti et al., 1995; Müller et al., 1997).

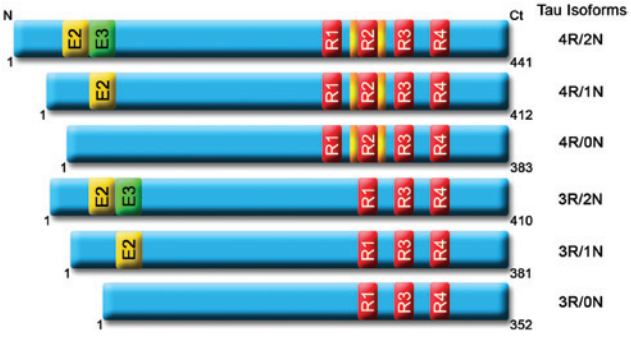

Figure 1: Schematic representation of the six human tau isoforms. The isoforms differ in the absence or presence of one or two inserts in the N-terminal region, encoded by exon 2 (yellow) and exon 3 (green). Three isoforms contain 3 microtubule binding repeats (R1, R3 and R4), and are named 3R-tau, and three contain 4 microtubule binding repeats (R1-R4), named 4R-tau. The fourth MT-binding repeat (R2) is encoded by exon 10.

Microtubules and the role of tau in oligodendrocytes

Oligodendrocytes have a complex morphological appearance; primary and secondary cellular processes are elaborated, which in culture form flat membranous sheets containing myelin specific proteins such as myelin basic proteins (MBP), proteolipidprotein (PLP), 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP), myelin-associated glycoprotein (MAG) and myelin oligodendrocyte glycoprotein (MOG) (Pfeiffer et al., 1993; Bradl and Lassmann, 2010). The oligodendrocyte architecture is maintained by an elaborate MT network (Figure 2), which is important for the establishment and maintenance of the metabolically active myelin membrane. MTs are highly dynamic and provide the tracks for organelle trafficking and intracellular transport processes (Richter-Landsberg, 2008; Bauer et al., 2009). Cargo is removed from the cell periphery and back (Ross et al., 2008). MBP mRNA is transported along the MTs to the distant regions and locally translated near the developing myelin sheath (Carson et al., 1997). In oligodendrocytes MTs occur in distinct subpopulations, in the fine processes MTs are more labile, while relatively stable MTs are present in the main branches (Lunn et al., 1997). A number of myelin specific proteins have been found to bind to MTs and specifically MBP, which is absolutely required for the formation of intact myelin, has been reported to function as a MT stabilizing protein in differentiated oligodendrocytes (Galiano et al., 2006; Richter-Landsberg, 2013). Furthermore a variety of MAPs, including high molecular weight MAPs and tau seem to be involved in the regulation of MT growth and stability (Müller et al., 1997; Richter-Landsberg, 2008).

In cultured oligodendrocytes derived from the brains of newborn rats all six tau isoforms are present and during *in vitro* differentiation tau heterogeneity increases while a decrease in phosphorylation is observed (Gorath et al., 2001). It has been implied that in adult rodent brain only 4R-tau isoforms are expressed. However, our data demonstrated that in rat brain 3R-tau isoforms are present yet at a much lower level (Müller et al., 1997; Gorath et al., 2001), which was later corroborated by Hanes et al. (2009). In cultured oligodendrocytes tau protein is developmentally regulated and 4R-isoforms become more prominent, while 3R-isoforms decrease (Seiberlich et al., 2015). Tau mRNA is actively transported to the cellular processes

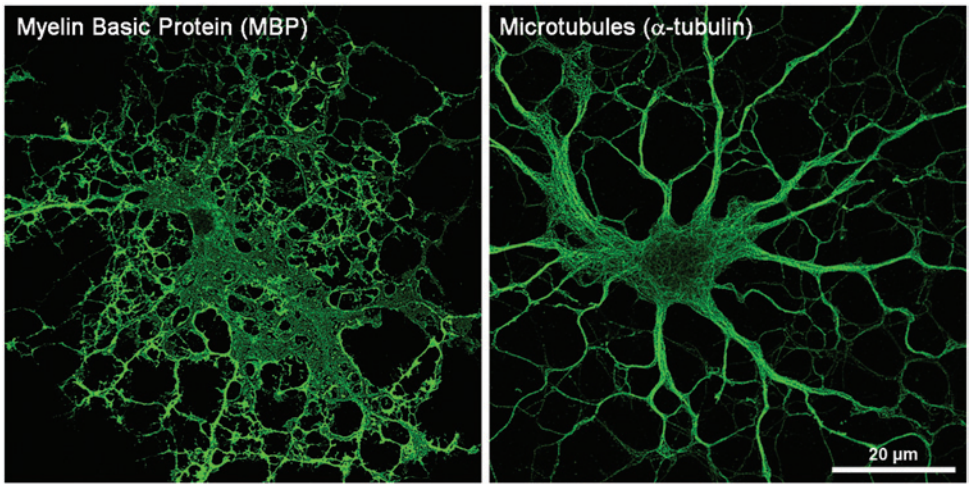

Figure 2: Oligodendrocytes are characterized by their elaborate microtubule network.
Oligodendrocytes derived from the brains of newborn rats after 5 days in culture were fixed and indirect immunofluorescence staining was carried out with antibodies against myelin basic protein (left) or α-tubulin (right). The picture was taken with a laser scanning confocal microscope.

and particularly present in the primary processes (Gorath et al., 2001). Oligodendroglial tau is phosphorylated at multiple sites identified by antibodies against AT-8, PHF-1 and 12E8. Phosphorylation is increased by okadaic acid treatment, which is an inhibitor of protein phosphatase 2A (PP2A) (Goldbaum and Richter-Landsberg, 2002). Under stress situations, such as oxidative stress or heat shock, tau is transiently dephosphorylated and rephosphorylation is mediated by glycogen synthase kinase 3β and suppressed by lithium chloride (Goldbaum and Richter-Landsberg, 2002). As dephosphorylation promotes better binding to and stabilization of MTs, this might be a protective means and a first line of defense against cellular stress.

The presence of tau is important for MT stability and MT formation. However, tau overexpression in oligodendrocytes has detrimental effects. Oligodendrocytes prepared from transgenic mice overexpressing human tau (wild type or the P301L mutation) under a CNP-promoter (Higuchi et al., 2005) were not viable for more than 5 days. MTs were extremely thin and defects in the MT network were observed (Richter-Landsberg, 2008). Conversely, downregulation of tau by siRNA technique impaired process outgrowth and the transport of MBP mRNA to the cell periphery (Seiberlich et al., 2015). In tau-deficient cells MBP expression was diminished and the expression of the proteoglycan NG2 enhanced. NG2 is a marker of oligodendrocyte precursor cells and its expression under normal conditions is downregulated during cell maturation (Nishiyama et al., 2014). Cells failed to differentiate into MBP-expressing, sheet-forming, mature oligodendrocytes, and MTs were not as arborized and less bundled. Additionally, these cells were not capable to properly myelinate axons when cocultured with neurons derived from dorsal root ganglions of newborn rats (Seiberlich et al., 2015). Hence a disturbance of the balance of tau leads to abnormalities in oligodendrocyte differentiation. The dynamics of MTs must be tightly regulated as has been suggested for neurons, otherwise they cannot fulfill their diverse functions. Overstabilization or understabilization of MTs, which might occur after misregulation of tau, may lead to a disturbance of the MT dynamic properties and to neurodegeneration (Feinstein and Wilson, 2005). In mice lacking tau protein a reduction in the number and density of MTs occurred (Harada et al., 1994). These mice showed an increased susceptibility to neuronal damage and more severe clinical disease in experimental autoimmune encephalomyelitis, which might relate to a defective oligodendroglial cytoskeleton and a decrease in MT stability (Weinger et al., 2012). The rather subtle phenotypes observed in tau knockout animals may be explained by an increase in the MAP 1A, which might compensate for the absence of tau.

Cellular stress and tau aggregate formation in oligodendrocytes

The formation of protein aggregates has been connected to an impairment or overload of the major protein degradation systems, the ubiquitin proteasome system (UPS) and the lysosomal degradation pathway (Schwartz and Ciechanover, 2009; Xilouri and Stefanis, 2011;

Richter-Landsberg and Leyk, 2013). For proteasomal degradation proteins are covalently modified by ubiquitin in a multistep procedure and delivered to the proteasome, which is the major system for the degradation for short-lived cytoplasmic proteins. Long-lived proteins, large protein aggregates, organelles and unwanted material are degraded by macroautophagy, hereafter referred to as autophagy (Klionsky et al., 2012; Richter-Landsberg and Leyk, 2013). The process is divided into several distinct steps: induction, cargo recognition and selection, autophagosome formation, and fusion of the autophagosome with the lysosome, followed by cargo degradation and release of the cargo into the cytosol. The detailed molecular mechanisms underlying these individual steps have been detailed in many excellent reviews and will not be summarized here (Johansen and Lamark, 2011; Klionsky and Schulman, 2014). Autophagy is the only degradation system for large protein aggregates, since these are excluded from the proteasome. It has emerged as a rather selective process that eliminates specific structures and organelles (Johansen and Lamark, 2011). Furthermore, heat shock proteins (HSPs), which act as molecular chaperones, participate in the defense against misfolded proteins. They assist in refolding of damaged proteins and target irreversibly damaged proteins to the UPS (Richter-Landsberg, 2007). HSPs are upregulated as a first line of defense against the accumulation of misfolded proteins, keep unfolded proteins in a competent state to be refolded, and may prevent cell death und maintain the cytoskeleton (Richter-Landsberg and Goldbaum, 2003). Proteasomal inhibition leads to HSP induction (Goldbaum and Richter-Landsberg, 2004), and the autophagosomal system can act as a compensatory mechanism when the UPS is impaired (Pandey et al., 2007; Jaenen et al., 2010). Several proteins have been identified, which link the UPS with the autophagic degradation system, including the receptors for ubiquitinated protein aggregates p62/sequestosome 1 and HDAC6 (histone deacetylase 6).

Protein aggregate formation is a dynamic process; large aggregates can be formed by the self-assembly of denatured monomeric proteins or oligomeric seeds. These can be present throughout the cytoplasm and grow into small aggregates. They may be delivered into specialized regions near the microtubule organizing center (MTOC) and form the so called aggresomes (Kopito, 2000), which share biochemical and molecular properties with inclusion bodies in neurodegenerative disorders. In cultured cells they can be located by the presence of γ-tubulin, which is a constituent of the MTOC (Richter-Landsberg, 2008). Aggresomes contain a variety of HSPs, ubiquitinated proteins, constituents of the proteasome and the autophagic pathway (Schwarz et al., 2012). The retrograde transport of small aggregates is mediated by dynein motor proteins and requires an intact cytoskeleton (Figure 3). Furthermore

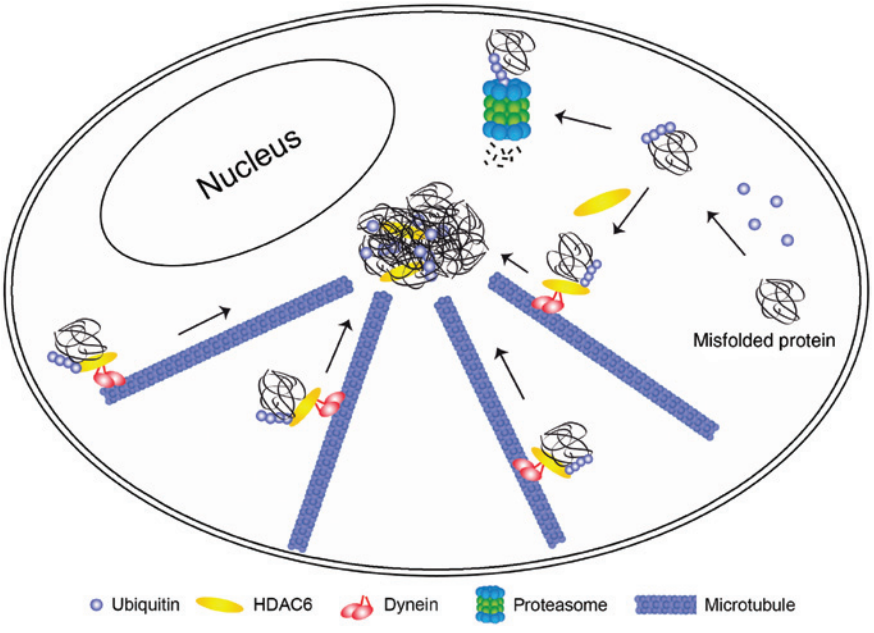

Figure 3: Aggresome formation is a dynamic process.
Misfolded ubiquitinated proteins are either degraded by the UPS or, when the UPS is impaired or overloaded, assemble as small aggregates in the periphery. HDAC6 promotes delivery of these ubiquitinated aggregates by a dynein-mediated retrograde transport along the microtubule tracks to the MTOC. Tubulin acetylation and the association of HDAC6 with the dynein motor protein enables this process.

ubiquitin-binding linker proteins are involved, including HDAC6 (see below). The process is disturbed by MT destabilizing drugs such as nocodazole (Bauer and Richter-Landsberg, 2006). Aggresomes sequester toxic oligomeric species and their formation enables efficient degradation of aggregated proteins by autophagy.

The timely sequence of aggregate or aggresome formation is difficult to follow/ in animal models. Hence, to study the molecular events accompanying protein aggregate formation, we have used primary rat brain oligodendrocytes and an oligodendroglial cell line, namely OLN-93 cells, which was developed in our laboratory (Richter-Landsberg and Heinrich, 1996). We have shown that proteasomal inhibition by MG-132 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal) in oligodendrocytes led to the ubiquitination of tau, to the upregulation of the small HSP αB-crystallin, its association with the MTs and tau, and to the appearance of tau-positive aggregates resembling inclusion bodies observed in tauopathies with glial pathology (Dabir et al., 2004; Goldbaum and Richter-Landsberg, 2004). Using OLN-93 cells, genetically engineered to stably express the longest human isoform of tau (4R2N), namely OLN-t40 cells, it was demonstrated that after tau hyperphosphorylation, induced by okadaic acid and proteasomal inhibition, stable fibrillary tau deposits were established (Goldbaum et al., 2003). In addition, non-fibrillary aggresomes in the vicinity of the MTOC were formed after proteasomal inhibition alone in a time and concentration-dependent manner (Bauer and Richter-Landsberg, 2006).

Ubiquitination is a reversible process, balanced by the actions of E3 ligases and deubiquitinating enzymes (DUBs). For proteasomal degradation the ubiquitin chains attached to the protein need to be removed before the substrate enters the proteolytic chamber of the proteasome. DUBs represent a large group of enzymes, which oppose the actions of E3 ligases, they maintain ubiquitin homeostasis, edit polyubiquitin chains, and prevent proteasomal ubiquitin degradation together with the substrates (Amerik and Hochstrasser, 2004; Komander et al., 2009; Todi and Paulson, 2011). Using PR-619, a cell permeable, broad-range inhibitor of deubiquitylases and ubiquitin-like isopeptidases (Altun et al., 2011), we could show that DUB inhibition in oligodendroglial OLN-t40 cells caused the time- and concentration-dependent induction of HSPs. Protein aggregates were formed, positively stained by antibodies against ubiquitin, αB-crystallin, HSP70 and p62, which increased in size over time and appeared as large deposits near the cell nucleus (Seiberlich et al., 2012). In addition, small tau aggregates were formed around the MTOC, but these did not colocalize with ubiquitin immunoreactivity. Besides the effect on protein aggregate formation, DUB inhibition by PR-619 also caused the alteration of the MT network. MTs appeared stabilized and more bundled, which resembled the effects of taxol, a MT stabilizing drug. This was not observed in OLN cells not expressing tau. Furthermore, DUB inhibition caused the inhibition of tau phosphorylation at the PHF-1 epitope, which increases its MT-binding capacity. This was sustained by an MT-binding assay, demonstrating that treatment with PR-619 enhanced the amount of tau binding to the MTs (Seiberlich et al., 2012). These effects most likely contribute to the observed stabilization and increased bundling of the MTs.

Taken together, the importance of the ubiquitin dependent pathways in the nervous system has become increasingly clear over the past years, and a loss of proteasomal function may contribute to neurodegenerative diseases and aging. In this respect DUBs have to be considered in pathological processes, as they are critically involved not only in balancing protein degradation pathways and protein aggregate formation, but also in maintaining the cellular architecture which is important for survival and functionality of neural cells (Seiberlich et al., 2012).

Tau acetylation and the role of HDAC6

Protein acetylation and deacetylation has emerged as a key regulatory mechanism, which is modified during a variety of stress situations. It plays a crucial role in pathological contexts including neurodegeneration, and is of importance in the regulation of autophagy (Trué and Matthias, 2012; Banreti et al., 2013). Acetylated tau was recently detected in glial and neuronal inclusions in tauopathies (Min et al., 2010; Cohen et al., 2011; Irwin et al., 2012). Tau acetylation at lysine 280 has been suggested to be a pathological modification contributing to tau-mediated neurodegeneration, as this modification inhibits its function and promotes pathological tau aggregation (Cohen et al., 2011). Impaired MT assembly and the promotion of tau fibrillization *in vitro* were observed, and enhanced acetylation of tau was shown to block its ubiquitination and turnover (Min et al., 2010).

Lysine acetylation and deacetylation was initially studied in histones, but histone acetylases (HATs) and histone deacetylases (HDACs) also have other non-histonal substrates, present in the nucleus and cytoplasm. Acetylation of tau in neurons is mediated by p300 acetyltransferase and CREB-binding protein (CBP) possibly at different sites

(Cohen et al., 2011; Cook et al., 2014). Experiments carried out by Min et al. (2010) suggested that the deacetylase sirtuin 1 (SIRT1), which is localized exclusively in neurons, may be involved in tau deacetylation. Other studies using antibodies against tau acetylated on K280, which recognize an epitope present in the second MT-binding repeat, pointed to the involvement of HDAC6, while SIRT2 was excluded (Cohen et al., 2011). SIRT2 is another HDAC with tubulin deacetylase activity and represents a major deacetylase in oligodendrocytes (Harting and Knoll, 2010).

HDAC6 is mainly localized in the cytosol, and many activities of HDAC6 are exerted through its modulatory effects on cytoskeletal proteins (for recent reviews see, Boyault et al., 2007; Li et al., 2011; Richter-Landsberg and Leyk, 2013). HDAC6 deacetylates substrates other than histones, in particular α-tubulin, cortactin and HSP90. α-Tubulin was the first HDAC6 substrate identified and HDAC6 is often referred to as a tubulin deacetylase. Acetylation of α-tubulin occurs mainly on polymerized microtubules at lysine 40, while deacetylation has been observed preferentially on depolymerized tubulin in a rapid process (Matsuyama et al., 2002). Human HDAC6 has unique structural features (Figure 4). It contains two deacetylase catalytic domains, i.e. DD1 and DD2, and two regions that are responsible for its localization: a domain responsible for its stable retention in the cytoplasm, i.e. SE14 (a Ser-Glu-containing tetradecapeptide), and a conserved nuclear exclusion domain NES, which prevents its accumulation in the nucleus. Furthermore, HDAC6 is characterized by the presence of a zinc-finger motif in the C-terminal end, which in its central part is similar to regions found in a variety of ubiquitin-specific proteases (ZnF-UBP). This domain enables HDAC6 to bind to mono- and poly-ubiquitinated proteins with a high specificity. Additionally, a dynein binding domain (DMB) is present. This region is essential for the dynein motor-based transport of ubiquitinated proteins along the microtubules to the aggresome (Kawaguchi et al., 2003). The various functions of HDAC6 depend on its deacetylase activity on the one hand and on its ubiquitin-binding capacity on the other hand. For

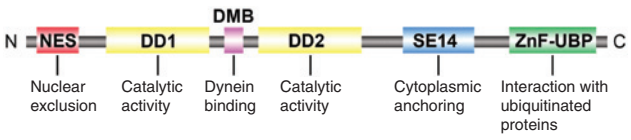

Figure 4: Schematic illustration of the functional domains in HDAC6. C, C-terminus; DD1 and DD2, catalytic domains 1 and 2; DMB, dynein binding domain; N, N-terminus; NES, nuc lear export signal; SE14, Ser-Glu-containing tetradecapeptide repeat domain; ZnF-UBP, zinc-finger ubiquitin-binding domain.

α-tubulin deacetylation the second domain of HDAC6 seems to be important. Increased acetylation of tubulin exerted by the treatment with tubacin, a specific inhibitor of DD2, resulted in increased acetylation of α-tubulin (Haggarty et al., 2003). This caused a slowdown in the rate of microtubule growth and shrinkage. Acetylation affects microtubule dynamics rather than microtubule stability, and acetylation of α-tubulin plays an important role in motor-based trafficking in mammalian cells. Kinesin-1 and dynein, proteins, which are involved in transport of cargos along the microtubule tracks, bind more effectively to acetylated microtubules, which leads to a stimulation of anterograde and retrograde transport processes (Perdiz et al., 2011).

Tau has been identified as an interaction partner of HDAC6. The association of HDAC6 with tau was shown in cell culture models and in human brain tissue, and was potentiated after proteasomal inhibition (Ding et al., 2008). HDAC6 interacts via its SE14 region with the MT-binding domain of tau, an interaction that is neither dependent on the deacetylase activity nor on the UBP binding domain of HDAC6. Also, tau co-immunoprecipitated with HDAC6 in control and AD brain extracts. Perez et al. (2009) demonstrated that an excess of tau binds to HDAC6 and decreases its deacetylase activity, and thus can act as an inhibitor of HDAC6.

We recently have shown that HDAC6 is present in oligodendrocytes, and involved in tau and α-tubulin deacetylation (Noack et al., 2014). Pharmacological inhibition of HDAC6 by the selective inhibitor tubastatin A led to an increase in α-tubulin acetylation, morphological alterations, MTs appeared more bundled and cellular processes were elongated, similarly in primary oligodendroytes and OLN-t40 cells (Figure 5). Treatment with tubastatin A caused an increase in tau acetylation, determined by the site specific acetylated K280 antibodies generated in the laboratory of Dr. Virginia Lee (Cohen et al., 2011), and a reduction in its turnover. Acetylation of tau occurred within the MT-binding domain. Concomitantly, tubastatin A caused an increase in phosphorylation of tau detected by the 12E8 antibody directed against Ser^{356} and Ser^{262}, which is located within the MT-binding domain and compromises the MT-binding activity of tau. Hence, HDAC6 inhibition and the concomitant tau hyperacetylation exerts a modifying effect on tau phosphorylation also in oligodendrocytes. Interestingly, HDAC6 inhibition in primary cultures of oligodendrocytes, which express all six tau isoforms, caused an increase in 4R-tau while 3R-tau isoforms were diminished. As in tauopathies with oligodendroglial pathology, i.e. PSP and CBD, 4R-tau pathology is prominent, this might be of special importance.

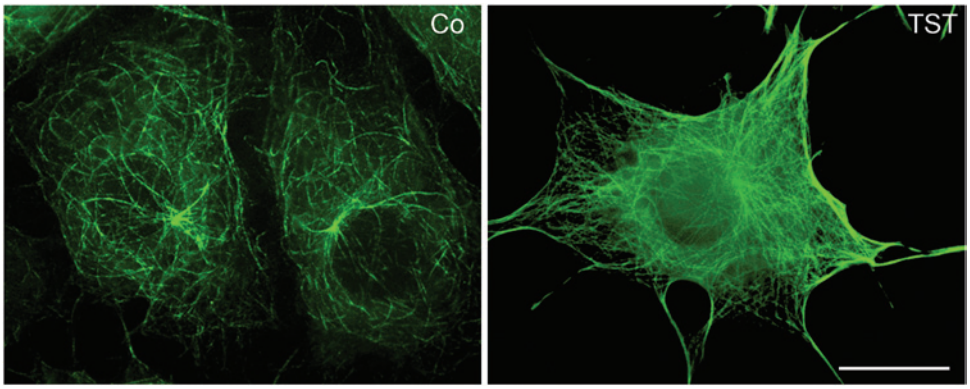

Figure 5: Effect of HDAC6 inhibition on microtubules in OLN-t40 cells.
OLN-t40 cells were treated with tubastatin A (TST, 10 μM for 19 h) and indirect immunofluorescence was carried out using antibodies against acetylated tubulin. MTs are heavily acetylated after treatment with TST and bundled. Co, Untreated control. Bar, 20 μm.

HDAC6, protein aggregate formation and autophagy

HDAC6 plays a central role in autophagy and aggresome formation. Aggresome formation is considered to be a protective means by segregating misfolded proteins with cytotoxic potentials from the cytoplasmic environment. This is particularly crucial for postmitotic cells like nerve cells and the myelin forming oligodendrocytes, because they lack the possibility to dilute protein accumulations by cell division. As mentioned above, while aggregates can assemble in the cell body and cellular processes of, for example, neurons and glia, aggresomes are formed in the perinuclear region at the MTOC and depend on coordinated transport processes. HDAC6 has been identified as a constituent of aggresomes, and cells deficient in HDAC6 are not capable of clearing protein aggregates from the cytoplasm and fail to form aggresomes (Kawaguchi et al., 2003; Iwata et al., 2005). HDAC6 binds to mono- and polyubiquitinated chains with a preference for K63-linked polyubiquitin chains, and mediates the retrograde transport of ubiquitinated proteins along the MT tracks, via its interaction with dynein motor proteins (Figure 3). Thus, HDAC6 functions as a bridge between MTs and polyubiquitinated proteins and is essential for their delivery to the growing aggresome, and for their subsequent autophagic degradation.

Another important role for HDAC6 is that it controls autophagosome maturation and in particular the fusion process of autophagosomes with lysosomes (Lee et al., 2010). This is achieved through activation of the actin-remodeling factor cortactin. The cytoplasmic protein cortactin is involved in the regulation of the structural dynamics of actin, it binds to filamentous actin (F-actin) and promotes actin polymerization and microfilament branching. Acetylation of cortactin impairs its interaction with F-actin. HDAC6 activates cortactin by deacetylation, and thus cortactin is recruited to the protein aggregates. Deacetylation of cortactin initiates F-actin polymerization. This promotes the assembly of a microfilament network, which is necessary for the fusion of the autophagosome with the lysosome and facilitates substrate degradation (Boyault et al., 2007; Banreti et al., 2013).

The knowledge about HDAC6 in oligodendrocytes and glial pathology is rather scarce. HDAC6 is a component of glial cytoplasmic inclusions in multiple system atrophy (Miki et al., 2011; Chiba et al., 2012), which is considered a primary oligodendrogliopathy, characterized by αB-crystallin deposits in oligodendrocytes (Jellinger and Lantos, 2010). As mentioned above, we have identified HDAC6 as a tubulin deacetylase present in oligodendrocytes and have shown that in oligodendrocytes α-tubulin and tau act as a substrate (Noack et al., 2014). In a recent study we have analyzed the involvement of HDAC6 in protein aggregate formation in oligodendrocytes. The data show that after proteasomal inhibition by MG-132 HDAC6 and acetylated tau are recruited to the growing aggregates, which contain other constituents of oligodendroglial lesions, such as ubiquitin and αB-crystallin (Leyk et al., 2015). After pharmacological inhibition of HDAC6 by tubastatin A, acetylated tau is more prominent in larger assemblies. Furthermore, after HDAC6 inhibition the assembly of MG-132-induced protein aggregates is altered, protein aggregates appear more dispersed throughout the cytoplasm. This is not a protective means, but promotes apoptotic cell death, supporting the conclusion that aggresome formation is a protective means and that smaller oligomeric species or seeds of proteins are rather toxic (Taylor et al., 2003; Richter-Landsberg and

Bauer 2004). In addition, our data demonstrate that inhibition of HDAC6 in oligodendrocytes leads to an increase in the formation of autophagic vesicles, but impairs the autophagic flux, which comprises the dynamic process of autophagosome formation, the delivery of substrates to the lysosomes, and their degradation (Leyk et al., 2015). Hence, HDAC6, which may be considered as a stress surveillance factor, contributes to stress responses and pathological processes in oligodendrocytes.

Concluding remarks

Oligodendrocyte differentiation and maturation is dependent on an intact, spatially organized cytoskeleton with dynamic properties. The myelinating cells of the CNS have an elaborate network of microtubules, which provides the tracks for organelle trafficking and intracellular translocation of myelin gene products including MBP mRNA. MAPs regulate the stability and growth of MTs. In this respect tau proteins are of importance. A loss of tau in oligodendrocytes disturbs MT stability and has severe consequences on oligodendrocyte differentiation, neuron-glia contact formation and the process of early myelination, while tau overexpression impairs MT formation and cell survival. A characteristic hallmark of many diseases is the abnormal accumulation of cytoskeletal and cellular proteins. Their occurrence may be related to the malfunctions of the proteolytic degradation systems, i.e. the proteasomal system and the autophagic pathway. A loss of tau functions occurs in tauopathies including AD, PSP and CBD, and microtubule stabilizing drugs are being discussed as therapeutic treatments to restore defects in microtubule stability and axonal transport (Ballatore et al., 2012). Protein acetylation and deacetylation may be modified in response to stress situations and has emerged as a key regulatory mechanism. Tau is a substrate for HDAC6 and acetylation of tau is a posttranslational modification with a pathological impact and represents a novel pathological signature in AD and other tauopathies (Irwin et al., 2012). Targeting the aggresome-autophagy pathway and HDAC6 is a promising avenue for therapeutic intervention, which may modify disease progression and promote cell survival.

Acknowledgments: This work was supported by the Deutsche Forschungsgemeinschaft (DFG RI 384/16-1 and 384/17-1). I thank Veronika Seiberlich and Janina Leyk for preparing the figures. All the present and past members of my laboratory are gratefully acknowledged for many years of fruitful collaborations and their contribution to our continuous efforts to elucidate the many roles of tau and the cytoskeleton in oligodendrocytes, which have fascinated me for more than 20 years.

References

Ahmed, Z., Bigio, E.H., Budka, H., Dickson, D.W., Ferrer, I., Ghetti, B., Giaccone, G., Hatanpaa, K.J., Holton, J.L., Josephs, K.A., et al. (2013). Globular glial tauopathies (GGT): consensus recommendations. Acta Neuropathol. *126*, 537–544.

Altun, M., Kramer, H.B., Willems, L.I., McDermott, J.L., Leach, C.A., Goldenberg, S.J., Kumar, K.G., Konietzny, R., Fischer, R., Kogan, E., et al. (2011). Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes. Chem. Biol. *18*, 1401–1412.

Amerik, A.Y. and Hochstrasser, M. (2004). Mechanism and function of deubiquitinating enzymes. Biochim. Biophys. Acta Bioenerget. *1695*, 189–207.

Ballatore, C., Lee, V.M., and Trojanowski, J.Q. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat. Rev. Neurosci. *8*, 663–672.

Ballatore, C., Brunden, K.R., Huryn, D.M., Trojanowski, J.Q., Lee, V.M. and Smith, A.B., 3rd. (2012). Microtubule stabilizing agents as potential treatment for Alzheimer's disease and related neurodegenerative tauopathies. J. Med. Chem. *55*, 8979–8996.

Banreti, A., Sass, M., and Graba, Y. (2013). The emerging role of acetylation in the regulation of autophagy. Autophagy *9*, 819–829.

Bauer, N.G. and Richter-Landsberg, C. (2006). The dynamic instability of microtubules is required for aggresome formation in oligodendroglial cells after proteolytic stress. J. Mol. Neurosci. *29*, 153–168.

Bauer, N.G., Richter-Landsberg, C., and Ffrench-Constant, C. (2009). Role of the oligodendroglial cytoskeleton in differentiation and myelination. Glia *57*, 1691–1705.

Boyault, C., Sadoul, K., Pabion, M., and Khochbin, S. (2007). HDAC6, at the crossroads between cytoskeleton and cell signaling by acetylation and ubiquitination. Oncogene *26*, 5468–5476.

Bradl, M. and Lassmann, H. (2010). Oligodendrocytes: biology and pathology. Acta Neuropathol. *119*, 37–53.

Carson, J.H., Worboys, K., Ainger, K., and Barbarese, E. (1997). Translocation of myelin basic protein mRNA in oligodendrocytes requires microtubules and kinesin. Cell. Motil. Cytoskeleton *38*, 318–328.

Chiba, Y., Takei, S., Kawamura, N., Kawaguchi, Y., Sasaki, K., Hasegawa-Ishii, S., Furukawa, A., Hosokawa, M., and Shimada, A. (2012). Immunohistochemical localization of aggresomal proteins in glial cytoplasmic inclusions in multiple system atrophy. Neuropathol. Appl. Neurobiol. *38*, 559–571.

Chin, S.S. and Goldman, J.E. (1996). Glial inclusions in CNS degenerative diseases. J. Neuropathol. Exp. Neurol. *55*, 499–508.

Cohen, T.J., Guo, J.L., Hurtado, D.E., Kwong, L.K., Mills, I.P., Trojanowski, J.Q., and Lee, V.M. (2011). The acetylation of tau inhibits its function and promotes pathological tau aggregation. Nat. Commun. *2*, 252.

Cook, C., Carlomagno, Y., Gendron, T.F., Dunmore, J., Scheffel, K., Stetler, C., Davis, M., Dickson, D., Jarpe, M., DeTure, M., et al. (2014). Acetylation of the KXGS motifs in tau is a critical determinant in modulation of tau aggregation and clearance. Hum. Mol. Genet. *23*, 104–116.

Dabir, D.V., Trojanowski, J.Q., Richter-Landsberg, C., Lee, V.M., and Forman, M.S. (2004). Expression of the small heat-shock protein alphaB-crystallin in tauopathies with glial pathology. Am. J. Pathol. *164*, 155–166.

Ding, H., Dolan, P.J., and Johnson, G.V. (2008). Histone deacetylase 6 interacts with the microtubule-associated protein tau. J. Neurochem. *106*, 2119–2130.

Dohm, C.P., Kermer, P., and Bahr, M. (2008). Aggregopathy in neurodegenerative diseases: mechanisms and therapeutic implication. Neurodegener. Dis. *5*, 321–338.

Feinstein, S.C. and Wilson, L. (2005). Inability of tau to properly regulate neuronal microtubule dynamics: a loss-of-function mechanism by which tau might mediate neuronal cell death. Biochim. Biophys. Acta *1739*, 268–279.

Galiano, M.R., Andrieux, A., Deloulme, J.C., Bosc, C., Schweitzer, A., Job, D., and Hallak, M.E. (2006). Myelin basic protein functions as a microtubule stabilizing protein in differentiated oligodendrocytes. J. Neurosci. Res. *84*, 534–541.

Gendron, T.F. and Petrucelli, L. (2009). The role of tau in neurodegeneration. Mol. Neurodegener. *4*, 13.

Goldbaum, O. and Richter-Landsberg, C. (2002). Activation of PP2A-like phosphatase and modulation of tau phosphorylation accompany stress-induced apoptosis in cultured oligodendrocytes. Glia *40*, 271–282.

Goldbaum, O. and Richter-Landsberg, C. (2004). Proteolytic stress causes heat shock protein induction, tau ubiquitination, and the recruitment of ubiquitin to tau-positive aggregates in oligodendrocytes in culture. J. Neurosci. *24*, 5748–5757.

Goldbaum, O., Oppermann, M., Handschuh, M., Dabir, D., Zhang, B., Forman, M.S., Trojanowski, J.Q., Lee, V.M., and Richter-Landsberg, C. (2003). Proteasome inhibition stabilizes tau inclusions in oligodendroglial cells that occur after treatment with okadaic acid. J. Neurosci. *23*, 8872–8880.

Gorath, M., Stahnke, T., Mronga, T., Goldbaum, O., and Richter-Landsberg, C. (2001). Developmental changes of tau protein and mRNA in cultured rat brain oligodendrocytes. Glia *36*, 89–101.

Haggarty, S.J., Koeller, K.M., Wong, J.C., Grozinger, C.M., and Schreiber, S.L. (2003). Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. Proc. Natl. Acad. Sci. USA *100*, 4389–4394.

Hanes, J., Zilka, N., Bartkova, M., Caletkova, M., Dobrota, D., and Novak, M. (2009). Rat tau proteome consists of six tau isoforms: implication for animal models of human tauopathies. J. Neurochem. *108*, 1167–1176.

Harada A., Oguchi K., Okabe S., Kuno J., Terada S., Ohshima T., Satoyoshitake R., Takei Y., Noda T., and Hirokawa N. (1994). Altered microtubule organization in small-calibre axons of mice lacking tau protein. Nature *369*, 488–491.

Harting, K. and Knoll, B. (2010). SIRT2-mediated protein deacetylation: an emerging key regulator in brain physiology and pathology. Eur J. Cell Biol. *89*, 262–269.

Higuchi, M., Zhang, B., Forman, M.S., Yoshiyama, Y., Trojanowski, J.Q., and Lee, V.M. (2005). Axonal degeneration induced by targeted expression of mutant human tau in oligodendrocytes of transgenic mice that model glial tauopathies. J. Neurosci. *25*, 9434–9443.

Himmelstein, D.S., Ward, S.M., Lancia, J.K., Patterson, K.R., and Binder, L.I. (2012). Tau as a therapeutic target in neurodegenerative disease. Pharmacol. Ther. *136*, 8–22.

Irwin, D.J., Cohen, T.J., Grossman, M., Arnold, S.E., Xie, S.X., Lee, V.M., and Trojanowski, J.Q. (2012). Acetylated tau, a novel pathological signature in Alzheimer's disease and other tauopathies. Brain *135*, 807–818.

Iwata, A., Riley, B.E., Johnston, J.A., and Kopito, R.R. (2005). HDAC6 and microtubules are required for autophagic degradation of aggregated huntingtin. J. Biol. Chem. *280*, 40282–40292.

Jaenen, S.B., Chaachouay, H., and Richter-Landsberg, C. (2010). Autophagy is activated by proteasomal inhibition and involved in aggresome clearance in cultured astrocytes. Glia *58*, 1766–1774.

Jellinger, K.A. (2009). Recent advances in our understanding of neurodegeneration. J. Neural. Transm. *116*, 1111–1162.

Jellinger, K.A. (2012). Interaction between pathogenic proteins in neurodegenerative disorders. J. Cell. Mol. Med. *16*, 1166–1183.

Jellinger, K.A. and Lantos, P.L. (2010). Papp-Lantos inclusions and the pathogenesis of multiple system atrophy: an update. Acta Neuropathol. *119*, 657–667.

Johansen, T. and Lamark, T. (2011). Selective autophagy mediated by autophagic adapter proteins. Autophagy *7*, 279–296.

Kawaguchi, Y., Kovacs, J.J., McLaurin, A., Vance, J.M., Ito, A., and Yao, T.P. (2003). The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. Cell *115*, 727–738.

Klein C., Kramer E.M., Cardine A.M., Schraven B., Brandt R., and Trotter J. (2002). Process outgrowth of oligodendrocytes is promoted by interaction of fyn kinase with the cytoskeletal protein tau. J. Neurosci. *22*, 698–707.

Klionsky, D.J. and Schulman, B.A. (2014). Dynamic regulation of macroautophagy by distinctive ubiquitin-like proteins. Nat. Struct. Mol. Biol. *21*, 336–345.

Klionsky, D.J., Abdalla, F.C., Abeliovich, H., Abraham, R.T., Acevedo-Arozena, A., Adeli, K., Agholme, L., Agnello, M., Agostinis P., Aguirre-Ghiso, J.A., et al. (2012). Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy *8*, 445–544.

Komander, D., Clague, M.J., and Urbe, S. (2009). Breaking the chains: structure and function of the deubiquitinases. Nat. Rev. Mol. Cell Biol. *10*, 550–563.

Kopito, R.R. (2000). Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol. *10*, 524–530.

Lee G., Newman S.T., Gard D.L., Band H., and Panchamoorthy G. (1998). Tau interacts with the src-family non receptor tyrosine kinases. J. Cell Sci. *111*, 3167–3177.

Lee, J.Y., Koga, H., Kawaguchi, Y., Tang, W., Wong, E., Gao, Y.S., Pandey, U.B., Kaushik, S., Tresse, E., Lu J., et al. (2010). HDAC6 controls autophagosome maturation essential for ubiquitin-selective quality-control autophagy. EMBO J. *29*, 969–980.

Leyk, J., Goldbaum, O., Noack, M., and Richter-Landsberg, C. (2015). Inhibition of HDAC6 Modifies Tau Inclusion Body Formation and Impairs Autophagic Clearance. J. Mol. Neurosci. *55*, 1031–1046.

Li, G., Jiang, H., Chang, M., Xie, H., and Hu, L. (2011). HDAC6 alpha-tubulin deacetylase: a potential therapeutic target in neurodegenerative diseases. J. Neurol. Sci. *304*, 1–8.

LoPresti, P., Szuchet, S., Papasozomenos, S.C., Zinkowski, R.P., and Binder, L.I. (1995). Functional implications for the microtubule-associated protein tau: localization in oligodendrocytes. Proc. Natl. Acad. Sci. USA *92*, 10369–10373.

Lunn, K.F., Baas, P.W., and Duncan, I.D. (1997). Microtubule organization and stability in the oligodendrocyte. J. Neurosci. *17*, 4921–4932.

Matsuyama, A., Shimazu, T., Sumida, Y., Saito, A., Yoshimatsu, Y., Seigneurin-Berny, D., Osada, H., Komatsu, Y., Nishino, N., Khochbin, S., et al. (2002). In vivo destabilization of dynamic microtubules by HDAC6-mediated deacetylation. EMBO J. *21*, 6820–6831.

Miki, Y., Mori, F., Tanji, K., Kakita, A., Takahashi, H., and Wakabayashi, K. (2011). Accumulation of histone deacetylase 6, an aggresome-related protein, is specific to Lewy bodies and glial cytoplasmic inclusions. Neuropathology *31*, 561–568.

Miller, D.W., Cookson, M.R., and Dickson, D.W. (2004). Glial cell inclusions and the pathogenesis of neurodegenerative diseases. Neuron Glia Biol. *1*, 13–21.

Min, S.W., Cho, S.H., Zhou, Y., Schroeder, S, Haroutunian, V., Seeley, W.W., Huang, E.J., Shen, Y., Masliah, E., Mukherjee, C., et al. (2010). Acetylation of tau inhibits its degradation and contributes to tauopathy. Neuron *67*, 953–966.

Morris M., Maeda S., Vossel K., and Mucke L. (2011). The many faces of tau. Neuron *70*, 410–426.

Müller, R., Heinrich, M., Heck, S., Blohm, D., and Richter-Landsberg, C. (1997). Expression of microtubule-associated proteins MAP2 and tau in cultured rat brain oligodendrocytes. Cell Tissue Res. *288*, 239–249.

Nishiyama, A., Suzuki, R., and Zhu, X. (2014). NG2 cells (polydendrocytes) in brain physiology and repair. Front. Neurosci. *8*, 133.

Noack, M., Leyk, J., and Richter-Landsberg, C. (2014). HDAC6 inhibition results in tau acetylation and modulates tau phosphorylation and degradation in oligodendrocytes. Glia *62*, 535–547.

Pandey, U.B., Nie, Z., Batlevi, Y., McCray, B.A., Ritson, G.P., Nedelsky, N.B., Schwartz, S.L., DiProspero, N.A., Knight, M.A., Schuldiner, O., et al. (2007). HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature *447*, 859–863.

Perdiz, D., Mackeh, R., Pour, C., and Baillet, A. (2011). The ins and outs of tubulin acetylation: more than just a posttranslational modification. Cell Signal. *23*, 763–771.

Perez, M., Santa-Maria, I., Gomez de Barreda, E., Zhu, X, Cuadros, R., Cabrero, J.R., Sanchez-Madrid, F., Dawson, H.N., Vitek, M.P., Perry, G., et al. (2009). Tau – an inhibitor of deacetylase HDAC6 function. J. Neurochem. *109*, 1756–1766.

Pfeiffer, S.E., Warrington, A.E., and Bansal, R. (1993). The oligodendrocyte and its many cellular processes. Trends Cell Biol. *3*, 191–197.

Richter-Landsberg, C. (2007). Heat shock proteins: expression and functional roles in nerve cells and glia. In: Heat Shock Proteins in Neural cells, C. Richter-Landsberg, ed. (New York: Landes Bioscience), pp. 1–24.

Richter-Landsberg, C. (2008). The cytoskeleton in oligodendrocytes. J. Mol. Neurosci. *35*, 55–63.

Richter-Landsberg C. (2013). Microtubules and associated proteins in oligodendrocytes, the myelin forming cells of the central nervous system. The cytoskeleton. Dermietzel R, ed. (New York: Springer), pp. 323–334.

Richter-Landsberg, C. and Bauer, N.G. (2004). Tau-inclusion body formation in oligodendroglia: the role of stress proteins and proteasome inhibition. Int. J. Dev. Neurosci. *22*, 443–451.

Richter-Landsberg, C. and Goldbaum, O. (2003). Stress proteins in neural cells: functional roles in health and disease. Cell. Mol. Life Sci. *60*, 337–349.

Richter-Landsberg, C. and Goldbaum, O. (2007). Small heat shock proteins and the cytoskeleton. In: Heat Shock Proteins in Neural Cells, C. Richter-Landsberg, ed. (New York: Landes Bioscience), pp. 13–24.

Richter-Landsberg, C. and Heinrich, M. (1996). OLN-93: a new permanent oligodendroglia cell line derived from primary rat brain glial cultures. J. Neurosci. Res. *45*, 161–173.

Richter-Landsberg, C. and Leyk, J. (2013). Inclusion body formation, macroautophagy, and the role of HDAC6 in neurodegeneration. Acta Neuropathol. *126*, 793–807.

Ross, J.L., Ali, M.Y. and Warshaw, D.M. (2008). Cargo transport: molecular motors navigate a complex cytoskeleton. Curr. Opin. Cell Biol. *20*, 41–47.

Schwartz, A.L. and Ciechanover, A. (2009). Targeting proteins for destruction by the ubiquitin system: implications for human pathobiology. Annu. Rev. Pharmacol. Toxicol. *49*, 73–96.

Schwarz, L., Goldbaum, O., Bergmann, M., Probst-Cousin, S., and Richter-Landsberg, C. (2012). Involvement of macroautophagy in multiple system atrophy and protein aggregate formation in oligodendrocytes. J. Mol. Neurosci. *47*, 256–266.

Seiberlich, V., Goldbaum, O., Zhukareva, V., and Richter-Landsberg, C. (2012). The small molecule inhibitor PR-619 of deubiquitinating enzymes affects the microtubule network and causes protein aggregate formation in neural cells: implications for neurodegenerative diseases. Biochim. Biophys. Acta *1823*, 2057–2068.

Seiberlich, V., Bauer, N.G., Schwarz, L., Ffrench-Constant, C., Goldbaum, O., and Richter-Landsberg C. (2015). Downregulation of the microtubule associated protein tau impairs process outgrowth and myelin basic protein mRNA transport in oligodendrocytes. Glia [Epub ahead of print]; doi: 10.1002/glia.22832.

Taylor, J.P., Tanaka, F., Robitschek, J., Sandoval, C.M., Taye, A., Markovic-Plese, S., and Fischbeck, K.H. (2003). Aggresomes protect cells by enhancing the degradation of toxic polyglutamine-containing protein. Hum. Mol. Genet. *12*, 749–757.

Todi, S.V. and Paulson, H.L. (2011). Balancing act: deubiquitinating enzymes in the nervous system. Trends Neurosci. *34*, 370–382.

Trué, O. and Matthias, P. (2012). Interplay between histone deacetylases and autophagy – from cancer therapy to neurodegeneration. Immunol. Cell Biol. *90*, 78–84.

Xilouri, M. and Stefanis, L. (2011). Autophagic pathways in Parkinson disease and related disorders. Expert Rev Mol Med. *13*, e8.

Weinger J.G., Davies P., Acker C.M., Brosnan C.F., Tsiperson V., Bayewitz A., and Shafit-Zagardo B. (2012). Mice devoid of tau have increased susceptibility to neuronal damage in myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis. J. Neuropathol. Exp. Neurol. *71*, 422–433.