Received: 15 October 2019 | Revised: 16 January 2020 | Accepted: 20 April 2020

DOI: 10.1002/dneu.22753

REVIEW ARTICLE

WILEY

Microtubule organization and dynamics in oligodendrocytes, astrocytes, and microglia

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Funding information
Dr. Miriam and Sheldon G. Adelson Medical Research Foundation; Vincent J. Coates Research Foundation; Myra Reinhard Foundation

Abstract

Though much is known about microtubule organization and microtubule-based transport in neurons, the development and function of microtubules in glia are more enigmatic. In this review, we provide an overview of the literature on microtubules in ramified brain cells, including oligodendrocytes, astrocytes, and microglia. We focus on normal cell biology—how structure relates to function in these cells. In oligodendrocytes, microtubules are important for extension of processes that contact axons and for elongating the myelin sheath. Recent studies demonstrate that new microtubules can form outside of the oligodendrocyte cell body off of Golgi outpost organelles. In astrocytes and microglia, changes in cell shape and ramification can be influenced by neighboring cells and the extracellular milieu. Finally, we highlight key papers implicating glial microtubule defects in neurological injury and disease and discuss how microtubules may contribute to invasiveness in gliomas. Thus, future research on the mechanisms underlying microtubule organization in normal glial cell function may yield valuable insights on neurological disease pathology.

KEYWORDS

astrocytes, cytoskeleton, glia, glioma, microglia, microtubules, oligodendrocytes

1 | INTRODUCTION

In polarized and ramified cells, the microtubule cytoskeleton is especially important for both structure and long-distance transport. The mammalian brain contains many ramified cell types, including neurons, oligodendrocytes, astrocytes, and microglia. Neurons, the electrically active cells that form the basis of systems-level circuits in the brain, contain two types of processes—generally a long axon and many shorter, branched dendrites. In neurons, much is known about microtubule polarity and organization (Kapitein & Hoogenraad, 2015; Nirschl, Ghiretti, & Holzbaur, 2017; Rao & Baas, 2018), microtubule-associated proteins (MAPs) and tubulin posttranslational modifications (PTMs) (Kelliher, Saunders, & Wildonger, 2019; Park & Roll-Mecak, 2018), as well as organelle interactions with microtubule-based motors, kinesins, and dynein (Fu & Holzbaur, 2014).

In this review, we focus on the less well-elucidated microtubule systems in glial cells in the brain—oligodendrocytes, astrocytes, and microglia. In vivo, these cell types have elaborate, ramified 3-dimensional (3D) structures. Thus, we focus on in vivo studies, including classic electron microscopy (EM) data as well as more recent two-photon microscopy studies. In vitro, many early experiments studied microtubules in glial-like cell lines or non-ramified primary cultures, but we focus on primary cultures that recapitulate the in vivo morphology of oligodendrocytes, astrocytes, and microglia. Finally, we contemplate outstanding questions on normal biology, including transport of glia-specific cargos, as well as on pathological changes observed in neurological injury and disease, including

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the recent discovery of microtubule-rich processes that contribute to invasiveness in glioma brain cancers.

2 | MICROTUBULES IN OLIGODENDROCYTES

The cellular architecture of oligodendrocytes is perhaps one of the most intriguing of any cell type in the body. Unlike Schwann cells, the myelinating cells of the peripheral nervous system (PNS), which make only one myelin sheath per cell, each oligodendrocyte in the central nervous system (CNS) is capable of making many myelin sheaths. During development, each oligodendrocyte extends many dynamic, branched processes that then contact axons and begin to make numerous myelin sheaths (Hughes, Kang, Fukaya, & Bergles, 2013). Estimates of the number of myelin sheaths produced per oligodendrocyte varies across brain regions, from ~15–25 in the cerebellum to ~25–40 in the cortex (Chong et al., 2012).

Microtubules in oligodendrocytes can morphologically be divided into two classes (Figure 1). Radial microtubules are proximal microtubules found along branched processes that extend toward the axon. Lamellar microtubules are distal microtubules that spiral longitudinally around the myelin sheath, starting from the outermost layer of myelin and reaching toward the innermost layers of myelin. Serial EM images elegantly demonstrated that lamellar microtubules reside inside cytoplasmic channels which resemble hollow tunnels that penetrate inside the myelin sheath (Snaidero et al., 2014). Myelin sheaths undergo compaction, a process by which cytoplasm is extruded by zippering together adjacent membranes that wrap around the axon in concentric layers (Aggarwal et al., 2011, 2013). Cytoplasmic channels are more common during developmental periods of myelination (25% of mice optic nerve sheaths at P10) and rarer in mature adult compact myelin (<5% at P60). Lamellar microtubules are functional and remarkably can mediate vesicular trafficking into inner myelin layers (Snaidero et al., 2014).

The formation of these cytoplasmic channels relies on 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP). This is consistent with earlier observations in cultured oligodendrocytes that CNP colocalizes with microtubules and is important for process outgrowth (Lee, Gravel, Zhang, Thibault, & Braun, 2005). In vivo, CNP null mice are nearly devoid of cytoplasmic channels. Mechanistically, CNP likely interacts with actin to form the "struts" or "dome" of the cytoplasmic channel. This was supported by cell-free experiments in which spherical giant unilamellar vesicles (GUVs) were dropped onto flat lipid bilayers. In the control, GUVs burst and merged with uncoated lipid bilayers. In contrast, when dropped onto lipid bilayers coated with CNP and actin, GUVs maintained their shape (Snaidero et al., 2017).

Microtubules in oligodendrocytes have uniform plus-ends-out polarity (Figure 1). In other words, the majority of microtubules grow away from the cell body. This has been demonstrated both by classic EM experiments using the hooking technique (Lunn, Baas, & Duncan, 1997) as well as by live-cell imaging of the plus-end microtubule-binding protein EB3 (Fu et al., 2019).

Recent experiments indicate that oligodendrocytes rely on nucleation, or the formation of new microtubules, at sites

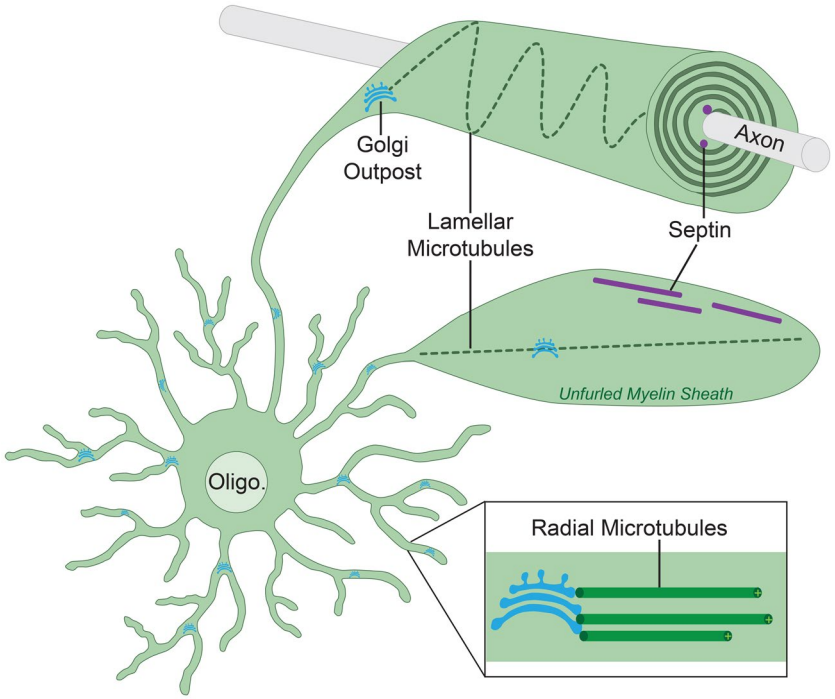

FIGURE 1 Microtubule Organization in Oligodendrocytes. Oligodendrocytes have radial (inset) and lamellar microtubules (dashed lines). Radial microtubules have uniform plus-ends-out polarity (inset). Golgi outposts (blue) are found along both radial and lamellar microtubules. Septin (purple) is found along the inner tongue of the myelin sheath, aligned longitudinally relative to the axon

outside of the cell body. Specifically, Golgi outposts are organelles found along oligodendrocyte processes and colocalize with the marker tubulin polymerization promoting protein (TPPP). In cell-free assays, TPPP is sufficient to nucleate microtubules. Loss of TPPP results in shorter lamellar microtubules and shorter myelin sheaths in 3D-cultured oligodendrocytes. In vivo, *Tppp* knockout mice also have shorter and thinner myelin sheaths and display defects in motor coordination on the Rotarod (Fu et al., 2019). These findings are consistent with an earlier study in which microtubule defects have been suggested to impair myelin sheath elongation in Schwann cells in the PNS (Court et al., 2004).

In addition to TPPP, oligodendrocytes also highly express other MAPs and contain microtubules with PTMs (Bauer, Richter-Landsberg, & Ffrench-Constant, 2009). These highly expressed MAPs include MAP2 and MAP4 (Vouyiouklis & Brophy, 1995) as well as MAP1B, which is thought to bundle proximal radial microtubules in premyelinating oligodendrocytes (Fischer, Konola, & Cochary, 1990; Vouyiouklis & Brophy, 1993). A specific MAP2 isoform is concentrated in MS lesions and may be involved in remyelination (Shafit-Zagardo, Kress, Zhao, & Lee, 1999). In cultured oligodendrocytes, stabilized acetylated microtubules are more enriched in the cell body and proximal processes, while tyrosinated microtubules are more enriched in distal processes (J. Song, Goetz, Baas, & Duncan, 2001).

The α-tubulin deacetylase SIRT2 (Sir-two-homolog 2) is expressed by both Schwann cells and oligodendrocytes (Southwood, Peppi, Dryden, Tainsky, & Gow, 2007). SIRT2 mediated destabilization of microtubules may function in lamellar microtubule retraction from the mature myelin sheath after membrane compaction has finished (Bauer et al., 2009). Spinal cord immuno-EMs find SIRT2 inside the myelin sheath, in the inner tongue (the noncompacted region directly apposed to the axon), and near the transmembrane protein PLP (proteolipid protein; Werner et al., 2007), which may indicate cotransport of SIRT2 into the myelin sheath via PLP-positive endosomes (Trajkovic et al., 2006). In cultured oligodendrocytes, SIRT2 knockdown increased α-tubulin acetylation and arbor complexity (Li et al., 2007). In contrast, in Schwann cells, selective ablation of SIRT2 in mice surprisingly did not change acetylated tubulin levels, but nevertheless resulted in delayed myelination, perhaps due to an additional SIRT2 target, the polarity kinase PAR3 (Beirowski et al., 2011). Thus, SIRT2 targets, including but not limited to acetylated tubulin, play important roles in myelination.

The geometry of the myelin sheath also relies on other cytoskeletal elements, including actin and septin. Actin depolymerization is important for myelin wrapping. Juvenile mice treated with latrunculin or lacking the actin depolymerizing factors ADF and cofilin have thinner myelin sheaths (Nawaz et al., 2015; Zuchero et al., 2015). Septin forms longitudinal filaments along the inner tongue (Figure 1), which is the innermost myelin sheath subcompartment that contains cytoplasm. In vivo, *Sept8* mutant mice have more myelin outfoldings, which are redundant myelin loops that are not surrounding an axon (Patzig et al., 2016). Thus, many cytoskeletal components contribute to building the myelin sheath and therefore the interplay between microtubules, actin, and septin and their coordination at different developmental time points will be of great future interest.

3 | MICROTUBULES IN ASTROCYTES

Astrocytes extend numerous processes that contact neuronal synapses as well as nearby capillaries. In the CNS, they secrete soluble factors and engulf synapses in order to modulate synaptic plasticity and transmission (Chung, Welsh, Barres, & Stevens, 2015).

In vivo, astrocyte morphology is impressively elaborate, with many branching processes. Classic EM images of rat optic nerve show that astrocyte processes contain intermediate filaments (IFs) and microtubules. At birth, astrocyte processes contain primarily microtubules, but during maturation, microtubules disappear almost entirely from the cytoplasm and are replaced by IFs (Peters & Vaughn, 1967). The subsequent discovery that a major component of astrocyte IFs is glial fibrillary acidic protein (GFAP) (Eng, Vanderhaeghen, Bignami, & Gerstl, 1971) led to the widespread use of GFAP as the principal marker for astrocytes (Figure 2).

More recently, in vivo visualization of microtubule dynamics in astrocytes was achieved using a genetically modified mouse expressing fluorescently tagged ensconsin microtubule-binding domain (EMTB). Time-lapse two-photon microscopy of the cortex showed that microtubules in astrocyte cell bodies can undergo extension, retraction, and branching events, but microtubules in processes are relatively stable and do not display major changes over 20-min imaging increments (Eom et al., 2011).

In vitro, primary astrocytes are typically cultured using two different methods: (1) the McCarthy-deVellis (MD)

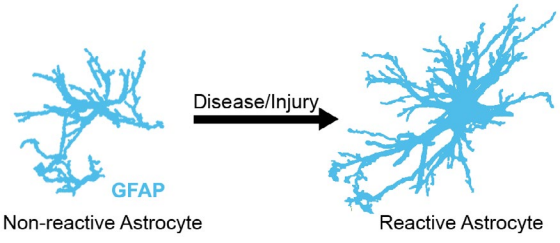

FIGURE 2 Reactive Astrocytes. Reactive astrocytes become more ramified. Astrocyte cartoon adapted from hippocampal astrocytes stained against GFAP following lesioning in the adjacent entorhinal cortex (Wilhelmsson et al., 2006)

method (McCarthy & de Vellis, 1980), and (2) the immunopanning method (Foo et al., 2011). MD astrocytes generally appear fibroblast-like with stubby protrusions. In both hippocampal and Purkinje neurons cultured with non-ramified glia, microtubule growth speed (visualized by EB3-GFP) is about twice as fast in glia compared to neurons (Stepanova et al., 2003); however, whether these glia are astrocytes and the mechanism underlying this speed difference is unclear.

In contrast, immunopanned astrocytes can develop long processes that resemble the in vivo architecture of astrocytes when cultured in the presence of several growth factors. Supplementing astrocyte cultures with fibroblast growth factors (FGFs) stimulates proliferation and increases long, branched processes (Pettmann, Weibel, Sensenbrenner, & Labourdette, 1985; Weibel, Fages, Belakebi, Tardy, & Nunez, 1987). A more recent study in immunopanned astrocytes found that bone morphogenic proteins (BMPs), when combined with FGF2 or heparin binding epidermal growth factor (HbEGF) promote a more process-bearing morphology (Scholze, Foo, Mulinyawe, & Barres, 2014). Consistent with these observations, in *Drosophila*, the FGF receptor Heartless is involved in the signaling that drives astrocyte process ramification (Stork, Sheehan, Tasdemir-Yilmaz, & Freeman, 2014).

A recent study showed that astrocyte morphology is affected by interactions with neurons. In vivo, in the Aldh1L1-EGFP mouse, a BAC (bacterial artificial chromosome) transgenic that expresses EGFP in astrocytes, morphogenesis of dense branching astrocyte processes in the visual cortex coincides with eye opening in the second week of life. Mice reared in the dark have decreased branching volumes, which suggests that astrocyte branching is affected by neuronal activity. In vitro, cortical rat astrocytes cultured in the absence or presence of neuron-conditioned media all have sparse branching. However, when cocultured with neurons, astrocytes develop their characteristic complex branching morphology. Thus, astrocyte morphology changes are not triggered by neuronally secreted factors, but, rather, by direct neuronal contact. This phenomenon requires neuroligin, a cell adhesion molecule that is expressed by astrocytes. Loss of neuroligin in vivo results in fewer astrocytic processes, fewer synapses within the astrocytic domain and altered synaptic function (Stogsdill et al., 2017).

The precise cytoskeletal changes underlying neuron-mediated morphology changes in astrocytes have yet to be elucidated. In vivo, microtubule labeling via EMTB (Eom et al., 2011) appear less arborized than GFP labeling of whole astrocytes in Aldh1L1-EGFP mice (Stogsdill et al., 2017). This implies that smaller, dense processes in astrocytes may be rich in actin or IFs. Indeed, a hybrid technique of staining dissociated cells from brain found that the dense, fine processes in astrocytes may be a meshwork of actin (Haseleu, Anlauf, Blaess, Endl, & Derouiche, 2013). Clearly, additional experiments are needed to understand the spatial organization of cytoskeletal elements in astrocytes.

4 | MICROTUBULES IN MICROGLIA

While neurons, astrocytes, and oligodendrocytes all derive from common neural stem cell (NSC) progenitors, microglia are myeloid cells that derive from the embryonic yolk sac. In vivo, microglia are ramified with dynamic branches that can contact synapses and survey their surroundings (Chung et al., 2015). Mice expressing GFP in lieu of the fractalkine receptor CX3CR1 (*CX3CR1*$^{GFP/+}$) have been used to study microglia morphology across different brain regions. For example, in the basal ganglia, microglia are heterogeneous in shape—cells from the nucleus accumbens and substantia nigra pars reticulata are more branched than those from the ventral tegmental area and substantia pars compacta (De Biase et al., 2017).

By two-photon microscopy, microglia in *CX3CR1*$^{GFP/+}$ mice are highly dynamic, extending and retracting their processes (Davalos et al., 2005; Nimmerjahn, Kirchhoff, & Helmchen, 2005). In the baseline condition, major processes (~2 μm in diameter) contain microtubules while minor processes (<0.5 μm in diameter) contain actin-rich filopodia. Minor processes are more dynamic than major processes, with average velocity of ~7–8 μm/min versus ~1 μm/min (Bernier et al., 2019). This is consistent with findings in retinal wholemounts that microglia processes colocalize with acetylated tubulin (Ilschner & Brandt, 1996), indicating that major processes may contain stabilized microtubules. In response to a small laser-induced injury (~6–15 μm in diameter), local microglia extend numerous processes toward the injury site in <10–30 min, without translocating their cell bodies (Davalos et al., 2005; Nimmerjahn et al., 2005). Pharmacological inhibitors pinpointed this response to be mediated by P2Y12 ligand-gated G protein-coupled receptors, which cause retraction of filopodia and extension of major processes toward the injury site (Bernier et al., 2019; Davalos et al., 2005).

Microglia may transition from an amoeboid state to a ramified state. Early EMs in postnatal brain found microglia are heterogeneous in shape—both amoeboid cells with filopodia and ramified cells with branches (Wu, Wen, Shieh, & Ling, 1994). Early culture experiments demonstrated that primary microglia transform from amoeboid to ramified when cocultured with primary astrocytes (Liu, Brosnan, Dickson, & Lee, 1994). This effect is likely due to an astrocyte secreted factor because primary microglia transform from amoeboid to ramified when cultured in serum-free media collected from astrocytes acutely stimulated for 30 min with lipopolysaccharide (LPS) (Ilschner

& Brandt, 1996). When antibodies against colony-stimulating factor (CSF-1) or transforming growth factor (TGF-β2) were added, microglia were less ramified (Liu et al., 1994; Schilling, Nitsch, Heinemann, Haas, & Eder, 2001). Recent studies defined that addition of three factors to serum-free media are sufficient to induce ramification of primary microglia—CSF-1, TGF-β2, and cholesterol. Removal of any of these three factors results in lack of ramified structures (Bohlen et al., 2017). The rationale for cholesterol addition came from RNA-seq data showing that microglia express low levels of cholesterol biosynthesis genes (Zhang et al., 2014), and therefore, may rely on other cells for their cholesterol supply (Bohlen et al., 2017). Thus, the development of ramified microglia in culture relies on growth factors that likely in vivo were secreted by astrocytes.

5 | OUTSTANDING QUESTIONS

Many basic questions remain on how oligodendrocytes, astrocytes, and microglia organize their cytoskeleton both in cell autonomous and non-cell autonomous manners. Both astrocytes and microglia develop more branched morphologies when cultured in the presence of neurons or astrocytes, respectively. Thus, cell–cell signaling can trigger changes in branching morphology. However, the downstream cytoskeletal targets underlying these structural changes stimulated by direct cell–cell contact or secreted factors have yet to be fully elucidated.

Below, we will address additional outstanding questions, including what specialized cargos may be transported in glia as well as how glial microtubules may be invovled in neurological injury, degeneration, and cancer.

5.1 | Transport of glia-specific cargos

In neurons, transport of cargos, such as mitochondria, autophagosomes, and synaptic vesicles have been a major focus of mechanistic studies. In glia, transport of different key cargos may also be important for cell health and function.

Oligodendrocytes transport cargos that are important for generating the myelin sheath and for communicating with neurons. They rely on transport of endosomes for delivery of transmembrane myelin proteins, such as PLP (Trajkovic et al., 2006), and of exosomes that participate in signaling with neurons (Fruhbeis et al., 2013). In addition, the myelin sheath is rich in mRNAs (Thakurela et al., 2016) and oligodendrocytes rely heavily on transport of myelin basic protein (*Mbp*) mRNA, which is the most highly expressed mRNA in oligodendrocytes (Zhang et al., 2014). *Mbp* mRNA is transported along microtubules so that it can be locally translated (Carson, Worboys, Ainger, & Barbarese, 1997; Lyons et al., 2009, Herbert et al., 2017).

Astrocytes also rely on transport of diverse cargos for cell-specific functions. Primary MD astrocytes display fast transport of lysosomes, excitatory amino acid transporter 1 (EAAT1), and the gap junction protein connexin 43 (Creighton, Ruffins, & Lorenzo, 2019). Interestingly, connexin 43 plays an important role in glioma cell invasiveness (Section 5.3) and knockdown decreases tumor area in xenografted mice (Osswald et al., 2015). Thus, understanding the normal transport of connexin 43 in astrocytes may yield crucial mechanistic insights on glioma proliferation and invasion.

In addition, astrocytes may also relie on mRNA transport for subcellular local translation. Primary astrocyte processes contain local mRNAs, including *Gfap*, ribosomal rRNA, and polyA mRNAs. The preferential localization of *Gfap* mRNA along astrocyte processes is disrupted by nocodazole depolymerization of microtubules (Medrano & Steward, 2001), indicating that *Gfap* mRNA is likely transported along microtubules. More recently, profiling of astrocyte processes using Boyden chambers and of astrocyte endfeet that contact blood vessels revealed a number of enriched local mRNAs (Boulay et al., 2017; Thomsen & Lade Nielsen, 2011), further suggesting that these mRNAs also may be transported along astrocyte processes.

5.2 | Glial microtubules in neurological disease

Glial microtubules may contribute to the etiologies of myelinating diseases, aggregating neurodegenerative diseases, neurodevelopmental disorders, and neural injury. Some of these contributions are summarized in Table 1.

In oligodendrocytes, distinct links between microtubules and myelin disease have been identified. Mutations in the β-tubulin gene *TUBB4A* cause leukodystrophy in children (Simons et al., 2013) and hypomyelination in the classic demyelinating *taiep* rat (Duncan et al., 2017). By RNA-seq, *Tubb4a* is the highest expressing β-tubulin mRNA in oligodendrocytes and its expression level in oligodendrocytes is higher than in other brain cell types by many fold (Zhang et al., 2014).

Expression levels of the oligodendrocyte Golgi outpost protein TPPP may be important in balancing a role in myelin repair versus aberrant aggregation. In multiple sclerosis (MS), TPPP is present in remyelinating lesions but absent in lesions that remain demyelinated (Hoftberger et al., 2010); this may indicate a role for local microtubule nucleation in myelin repair. Conversely, several histology studies have observed TPPP aggregates in Alzheimer's disease (Frykman et al., 2012) as well as in α-synucleinopathies, including

Parkinson's disease (Kovacs et al., 2004), Lewy body dementia (Lindersson et al., 2005), and multiple system atrophy (Song et al., 2007).

Upon injury to the CNS or in various neurodegenerative diseases, astrocytes become reactive with drastically altered morphology (Figure 2). This process of reactive astrogliosis involves hypertrophy of processes and accumulation of cells to form a glial scar, which can paradoxically both protect the injury site and hinder repair (Liddelow & Barres, 2017). Dye filling and 3D reconstruction techniques have demonstrated that hypertrophy in reactive astrocytes is confined to IF-rich processes while the overall astrocyte domain remains unchanged (Wilhelmsson et al., 2006). Reactive astrocytes have increased expression of GFAP, other IF proteins, such as vimentin and nestin (Wilhelmsson et al., 2004), as well as MAP2 (Geisert, Johnson, & Binder, 1990). Astrocytes from GFAP and vimentin double knockout mice are able to form processes during development, but do not show signs of process hypertrophy in disease states (Wilhelmsson et al., 2004), indicating that IFs are crucial for astrogliosis. Thus, understanding the roles played by IFs and MAPs in astrogliosis will be crucial to understanding neural injury response.

The development of astrocyte process morphology and branch elaboration may have links to neurodevelopmental disorders. Neuroligins, cell adhesion molecule that mediate astrocyte ramification (Stogsdill et al., 2017), are mutated in autism (Singh & Eroglu, 2013) and schizophrenia (Sun et al., 2011). Rett syndrome is a neurodevelopmental disorder caused by mutations in the methyl-CpG-binding protein 2 (*MECP2*) gene. Both cultured MD astrocytes from *Mecp2*-deficient mice and iPSC-derived astrocytes from human Rett patients have faster microtubule growth velocities and higher percentages of motile Lysotracker-positive vesicles (Delepine et al., 2016).

The MAP tau aggregates in neurons in Alzheimer's disease, but also in astrocytes and oligodendrocytes in corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP; Gibbons, Lee, & Trojanowski, 2019). In addition, mutation of tau causes globular glial tauopathy (Tacik et al., 2015). In cultured rat MD astrocytes, overexpression of human tau resulted in a reduction in detyrosinated microtubules and kinesin motors, leading to defects in organelle trafficking and eventual cell death (Yoshiyama, Zhang, Bruce, Trojanowski, & Lee, 2003). Mice that specifically express mutant human tau in oligodendrocytes initially display impaired axonal transport, followed by motor deficits and tau inclusions inside oligodendrocytes (Higuchi et al., 2005). The progression of pathological tau across different brain regions is thought to be driven by cell–cell interactions (Gibbons et al., 2019). A mouse model in which tau is knocked down in neurons suggests that this propagation may proceed along white matter tracts via oligodendrocytic tau, but not astrocytic tau (Narasimhan et al., 2020). Thus, aberrant tau complexly exerts both cell autonomous and non-cell autonomous pathological effects in neurons, oligodendrocytes, and astrocytes.

In conclusion, many complex interactions affecting glial microtubules may contribute to neurological disease (Table 1). Thus, understanding how cells in the brain communicate with each other to modify their morphology and how key cargos

TABLE 1 Glial microtubule proteins/genes implicated in neurological disease

Protein/Gene	Neurological disease	Glial effect	References
MAP2	Multiple sclerosis (MS) involvement	An isoform is enriched in oligodendrocytes in remyelinating lesions	Shafit-Zagardo et al. (1999)
MECP2	Rett syndrome mutation	Increases astrocyte microtubule growth speed and % motile Lysotracker-positive vesicles	Delepine et al. (2016)
NL3 (Neuroligin)	Autism and schizophrenia mutation	NL3-depleted astrocytes have fewer processes	Stogsdill et al. (2017)
Tau (*MAPT* gene)	Globular glial tauopathy (GGT) mutation	K317N mutation	Tacik et al. (2015)
	Corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP) pathology	Aggregates in oligodendrocytes and astrocytes; tau inclusions spread via white matter tracts and oligodendrocytes	(Narasimhan et al., 2020)
TPPP	Multiple system atrophy (MSA), Lewy body dementia, Parkinson's disease pathology	Aggregates	Kovacs et al., (2004), Lindersson et al. (2005), Song et al. (2007)
	Alzheimer's disease pathology	Aggregates	Frykman et al. (2012)
	Multiple sclerosis (MS)	Remyelination marker	Hoftberger et al. (2010)
TUBB4A	Leukodystrophy mutation	Enriched in oligodendrocytes	Duncan et al., 2017, Simons et al. (2013)

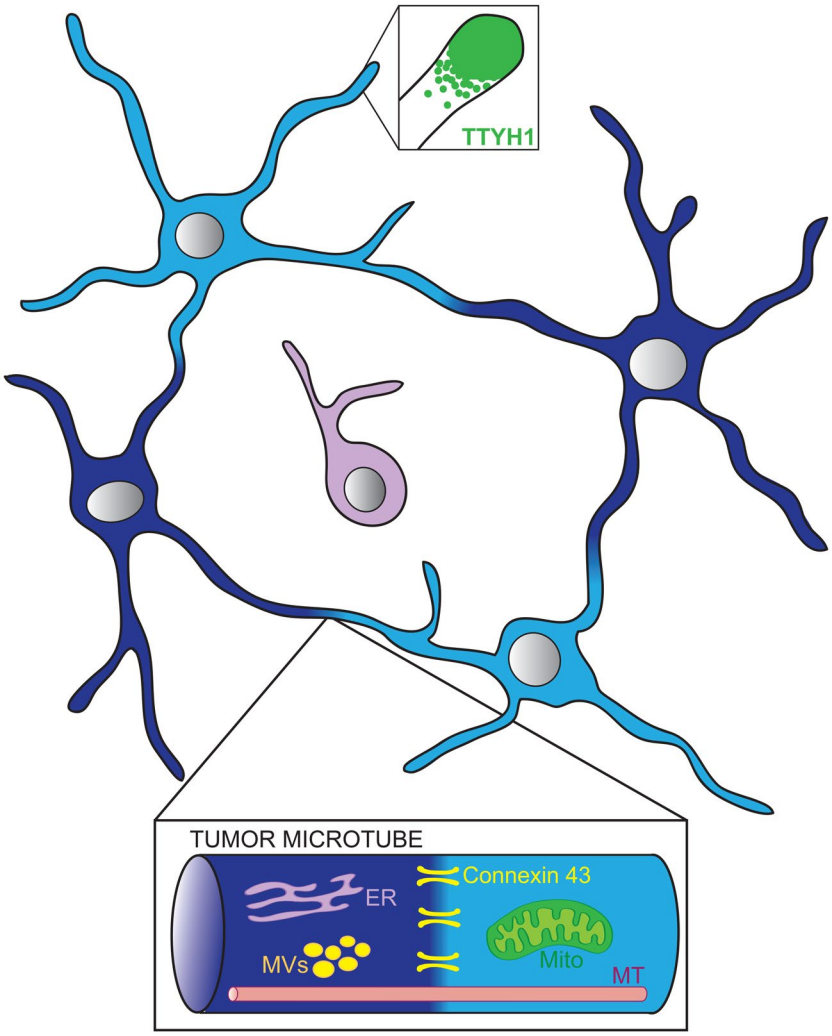

FIGURE 3 Tumor Microtubes in Glioma. Glioma tumor microtubes (TMs) are protrusions or extensions that are connected by gap junctions. They can be electrically active and synapse with neurons. Glioma cells with >4 protrusions are depicted in light and dark blue; a glioma cell with 1–2 protrusions is depicted in purple. (Top inset) The growth-cone-like tips of TMs are enriched in the transmembrane protein TTYH1, which is a marker for invasive TM-positive gliomas. (Bottom inset) TMs projecting from individual cells are connected by gap junctions, specifically connexin 43. TMs are rich in actin, microtubules (MT), and organelles, including endoplasmic reticulum (ER), mitochondria (Mito), and microvesicles (MVs)

contribute to glial cell function will be crucial to understanding the role of non-neuronal brain cells in disease and injury.

5.3 | Microtubules in Gliomas

Gliomas are a prevalent type of brain tumor that are highly invasive. Glioblastoma multiforme (GBM), a grade IV astrocytoma, is considered incurable with median survival of only 15 months (Davis, 2016; Thakkar et al., 2014).

Pivotal work in recent years has revealed that glioma cells are interconnected through membranous protrusions called tumor microtubes (TMs). TMs contain actin, microtubules, and organelles, including endoplasmic reticulum (ER) and mitochondria (Figure 3). Though most TMs in oligodendrogliomas are <50 μm long, many TMs in astrocytomas surpass lengths of >100 μm, with some reaching extraordinary lengths of >500 μm. TMs from different cells are connected by gap junctions, and knockdown of the gap-junction protein connexin 43 in tumors leads to reduced tumor sizes and improved survival in xenografted mice (Osswald et al., 2015).

A follow-up microarray screen identified tweety-homolog 1 (TTYH1) as an important driver of TM development and a marker for invasive TM-positive tumor cells (Jung et al., 2017). TTYH1 is a 5-pass transmembrane protein that when overexpressed in HEK293 cells leads to formation of copious filopodia-like protrusions (Matthews et al., 2007). In gliomas, TTYH1 is found along TM protrusions and concentrated at their growth-cone-like tips (Figure 3, top inset). TTYH1-positive glioma cells are highly motile and extend 1–2 TMs; in contrast, TTYH1-negative cells are relatively stationary and extend >4 TMs; these subpopulations may represent two stages of tumor progression—an initial invasion phase and a colonization phase, respectively. Thus, *Ttyh1* knockdown preferentially depleted glioma cells with 1–2 TMs, but not cells with >4 TMs and led to fewer tumor cells overall. In vivo, mice implanted with *Ttyh1*-depleted tumor cells have prolonged survival compared to those implanted with control tumor cells (Jung et al., 2017).

Two recent studies remarkably demonstrated that glioma TMs are electrically active and synapse with neurons. Blocking glioma electrical activity inhibits glioma growth (Venkataramani et al., 2019; Venkatesh et al., 2019). Thus,

the microtubules inside TM networks may be feasible molecular target for glioma therapies aimed at arresting TM growth or inhibiting transport along TMs. Though microtubule targeting agents have been widely used to treat different cancers (Mukhtar, Adhami, & Mukhtar, 2014; Schaefer, 2007), many are not permeable across the blood-brain barrier. The mechanism of action for these drugs are typically to disrupt microtubule dynamics, which leads to mitotic spindle damage and induces apoptosis. More specifically, these drugs can be divided into: 1) destabilizers (e.g. colchicine) that suppress microtubule polymerization and 2) stabilizers (e.g. paclitaxel) that increase microtubule polymerization (Jordan, 2002; Mukhtar et al., 2014). However, in light of recent findings, stabilization of TM microtubules may aid transport and hence have potentially deleterious roles. Alternatively, TM-associated MAPs may allow for more specific therapeutic targeting. Indeed, the microtubule-severing protein spastin may play a role in tumor invasiveness (Draberova et al., 2011), but it is not known whether spastin has a direct role in TM formation. Thus, it is more important than ever to understand the basic cell biology underlying gliomas and TMs.

In conclusion, glial microtubules have important roles in establishing the ramified morphology of oligodendrocytes, astrocytes, and microglia as well as in facilitating transport of key cell-specific cargos. However, many fundamental and outstanding questions remain on how glial microtubules form, how they interact with other cytoskeletal elements, how they are regulated, and how they contribute to transport. Thus, timely investigation of these topics may yield crucial insights to the roles of glial microtubules in development and in disease.

ACKNOWLEDGMENTS

We are grateful for funding from the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation, the Vincent J. Coates Research Foundation, and the Myra Reinhard Foundation (to Ben A. Barres).

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How to cite this article: Weigel M, Wang L, Fu M-M. Microtubule organization and dynamics in oligodendrocytes, astrocytes, and microglia. *Develop Neurobiol*. 2020;00:1–11. https://doi.org/10.1002/dneu.22753