Published in final edited form as:

J Neurosci Res. 2018 July ; 96(7): 1125–1135. doi:10.1002/jnr.24231.

Clinical Relevance of Terminal Schwann Cells: An Overlooked Component of the Neuromuscular Junction

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Abstract

The terminal Schwann cell (tSC), a type of non-myelinating Schwann cell (SC), is a significant yet relatively understudied component of the neuromuscular junction (NMJ). In addition to reviewing the role tSCs play on formation, maintenance, and remodeling of the synapse, we review studies that implicate tSCs in neuromuscular diseases including spinal muscular atrophy (SMA), Miller-Fisher syndrome, and amyotrophic lateral sclerosis (ALS) among others. We also discuss the importance of these cells on degeneration and regeneration after nerve injury. Knowledge of tSC biology may improve our understanding of disease pathogenesis and help us identify new and innovative therapeutic strategies for the many patients who suffer from neuromuscular disorders and nerve injuries.

Keywords

Glia; Neuromuscular Junction; Schwann cells

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Conflicts of Interest

The authors declare no known conflicts of interest.

Role of Authors

K.B.S. and A.K.S.W. conceptualized the scope of the paper, and K.B.S. and A.M.K. wrote the original draft of the manuscript. A.J.S. was responsible for the investigation and visualization/data presentation of the experimental IHC images. B.V. contributed to visualization/data presentation by creating the drawings used. All authors provided critical revisions of the manuscript for important intellectual content, and take responsibility for the accuracy and integrity of the manuscript. A.K.S.W. supervised the creation of this review manuscript.

Introduction

Over the past several decades, significant advances have been made in our understanding of the roles glial cells play in nervous system development, function, disease, and recovery after injury. Glial cells outnumber neurons, accounting for more than half of all cells in the mammalian nervous system (Zuchero and Barres 2015). Although they were previously thought only to passively support neuronal function, further investigation of these dynamic cells have shown that glia are integral to the development, function, and plasticity of the central and peripheral nervous systems, (Barres 2008; Fields et al. 2015) and play important roles in inflammation and disease (Casano and Peri 2015; Zuchero and Barres 2015).

Schwann cells (SCs) are the main glial cells of the peripheral nervous system (PNS) and can be subdivided into two types: myelinating and non-myelinating. Both SC subtypes arise from a common precursor, the immature SC (FIGURE 1). In contrast to myelinating SCs, which ensheath axons to allow efficient electrical impulse propagation, significantly less is known about non-myelinating SCs. There are three types of non-myelinating SCs: Remak SCs, specialized sensory transducers (such as Pacinian corpuscles), and tSCs, which differ in structure and location. tSCs (also known as perisynaptic Schwann cells or teloglia), are non-myelinating SCs that reside at the neuromuscular junction (NMJ), and are the focus of this review

The NMJ is a synapse that transmits signals from motor neurons and their axons to the target muscle. It is composed of four main elements: (1) presynaptic terminal nerve; (2) synaptic basal lamina that occupies the synaptic cleft; (3) post-synaptic region of the myofiber rich in acetylcholine receptors (AChRs); and (4) tSCs (FIGURE 2). Depending on muscle fiber type (slow or fast twitch), there are typically 1–3 tSCs per NMJ that are connected by cytoplasmic extensions, although the higher number of 3–5 tSCs per NMJ have been historically described (Balice-Gordon and Lichtman 1990). Immunohistochemically, tSCs can be easily identified by staining with S100b antibody, a marker of all SCs, both myelinating and non-myelinating (FIGURE 2). While relatively less is known about tSCs as compared to the other NMJ components, they are integral contributors to the development, maintenance, and regeneration of the synapse (Feng and Ko 2008; Kang et al. 2014; Macleod et al. 2001; O'Malley et al. 1999; Reist and Smith 1992; Reynolds and Woolf 1992; Son and Thompson 1995a; Son and Thompson 1995b; Son et al. 1996).

tSCs in NMJ Synaptogenesis

Although not necessary for initial NMJ formation, tSCs play a vital role in the growth and maintenance of the synapse during development (Darabid et al. 2014; Kullberg et al. 1977; Lin et al. 2000; Linden and Letinsky 1988; Woldeyesus et al. 1999). Herrera et al. studied the roles that tSCs play in amphibian NMJ development (Herrera et al. 2000). Their results demonstrate that in the first phase of amphibian NMJ development, tSCs occupy most NMJs, but only partially cover the synapse. In the second stage, tSCs begin to extend cytoplasmic processes, and numbers of tSCs at the NMJ increase. During this phase, the presynaptic nerve terminal is tightly apposed to AChRs on the end-target muscle and, muscle fibers proliferate, but there is no evidence of NMJ or muscle growth. tSCs then

maximally extend processes and cover all synaptic elements in the next stage, while NMJ length (referring to AChR labelling of synaptic gutters) increases. New NMJs do not initiate growth until full tSC occupation and cytoplasmic process extension is present. In phase 4, tSC cytoplasmic processes decrease as muscle fiber growth decreases and NMJ growth continues. By the 5th stage, the NMJ has achieved its adult form. tSC process extension is rare (Herrera et al. 2000).

In rat NMJ development, half of the NMJs are covered by one tSC, and the other half are covered only by SC processes (Love and Thompson 1998). The fastest rate of tSC gain at the NMJ occurs within the first two postnatal weeks, preceding the maturation of the motor endplate. tSCs are gained via both mitosis and migration, and their final number per NMJ depends on the specific muscle. During the period of poly-innervation of the developing NMJ (discussed below), an average of 1.6 tSCs are present at the NMJ, compared to 2.7 tSCs per NMJ during the mono-innervation phase (Darabid et al. 2013). By 2 months of age, tSC processes have retracted, and 2–3 tSCs cap the NMJ (Love and Thompson 1998).

In a process termed synaptic elimination, tSCs directly compete with the receptive surface of the end-target muscle to transition from polyneuronal innervation, in which multiple presynaptic motor neurons contact the AChRs at one motor endplate, to mononeuronal NMJ innervation. At P7–8 in murine NMJ development, more than 60% of NMJs are poly-innervated (Darabid et al. 2013). tSCs extend finger-like processes between adjacent nerve terminals and between the nerve terminals and muscle fibers, competing for AChR binding sites. Subsequently, tSCs randomly phagocytose nerve terminals that contact AChRs, promoting mononeuronal innervation (Smith et al. 2013). Single tSCs have also been shown to decipher the strength of individual synaptic activity during synaptic competition and influence synaptic elimination (Darabid et al. 2013). Synaptic elimination has been noted to occur at the period of greatest tSC occupation at the NMJ in amphibians (Herrera et al. 2000) and mammals (Love and Thompson 1998). A recent study supports these findings and suggests that tSC phagocytic roles are induced by neuronal expression of Neuregulin1 (Lee et al. 2016).

Interruption or loss of any NMJ component during development is detrimental. Neonatal NMJ denervation results in tSC apoptosis (Trachtenberg and Thompson 1996). When tSCs are ablated in amphibians during NMJ development, nerve terminals retract, synaptic addition and growth decreases, and the synapse retracts (Reddy et al. 2003). However, when GGF2, the soluble form of neuregulin (NRG), is applied to tSCs after NMJ denervation in development, cells are rescued from apoptosis (Trachtenberg and Thompson 1996). NRGs are trophic factors that are present in developing motor neurons and have been shown to bind erbB3 receptors expressed on tSCs to prevent apoptotic death of SC precursors, stimulate SC DNA synthesis, and support long-term survival of SC precursors (Dong et al. 1995; Lin et al. 2000; Mahanthappa et al. 1996; Trachtenberg and Thompson 1996; Trinidad et al. 2000). These findings provide evidence that tSCs are necessary for NMJ stability during development and synaptic elimination during synaptogenesis. Furthermore, these studies suggest that trophic interactions are important to NMJ development.

tSCs in Synaptic Modulation and Plasticity

Multiple studies have demonstrated that tSCs sense synaptic activity and, in response, modulate synaptic transmission to ensure appropriate signal strength and efficacy at the NMJ. The first of these studies were published in 1992 by Jaromi et al. and Reist and Smith, revealing that tSCs respond to synaptic activity. Both groups found that following presynaptic nerve stimulation at amphibian NMJs, there is increased intracellular calcium in tSCs. This rise in tSC intracellular calcium is blocked when neurotransmitter release from presynaptic nerves is blocked (Jahromi et al. 1992; Reist and Smith 1992). Additionally, trains of nerve stimulation result in smaller calcium responses in tSCs (Jahromi et al. 1992; Reist and Smith 1992). These findings are similar to studies performed at mammalian tSCs, although mammalian tSCs display the calcium-dependent calcium-release phenomenon that is absent in amphibian NMJs (Rochon et al. 2001). Subsequent studies performed by Robitaille's group revealed that the neurotransmitters released by the presynaptic nerve that are responsible for the increase in tSC intracellular calcium are: adenosine triphosphate (ATP); its degradation product, adenosine; acetylcholine (ACh); and substance P (SP) (Bourque and Robitaille 1998; Georgiou et al. 1999; Robitaille 1995; Robitaille et al. 1997). These studies were conducted in amphibians, but many of the same findings were corroborated, with slight variations, in mammalian studies (Rochon et al. 2001). One key difference between the mammalian and amphibian NMJs is the neurotransmitter receptors expressed by tSCs. Mammalian tSCs express muscarinic acetylcholine receptors (mAChR) that are atropine-sensitive, A1 receptors for adenosine, and an unidentified, suramin-insensitive, purinergic receptor (Rochon et al. 2001). Recent studies have also suggested the possibility of P2y receptor expression on mammalian tSC during development, but not adulthood (Arbour et al. 2015; Darabid et al. 2013). tSCs express mAChRs that are atropine-insensitive and, therefore, are not of the typical M1–M5 phenotype. Additionally, amphibian tSCs express A1 receptors for adenosine and P2x and P2y receptors for ATP (Robitaille 1995; Robitaille et al. 1997; Rochon et al. 2001).

In addition to sensing synaptic transmission, tSCs play an important role in modulating synaptic activity. Using amphibian NMJs, it has been shown that tSC G-protein activation results in synaptic depression and decreased neurotransmitter release from the presynaptic nerve (Robitaille 1998). Furthermore, synaptic depression initiated by the tSCs is not continuous, but rather occurs during high frequency presynaptic nerve activity. These findings suggest that tSCs sense variations in synaptic activity, and are able to modulate synaptic transmission, with the ultimate effect of decreasing the efficacy and strength of the synapse in response to high frequency stimulation (Robitaille 1998).

tSCs may modulate synaptic potentiation or depression, based on the synaptic activity profile. In response to continuous presynaptic nerve stimulation, tSCs respond with a phasic calcium response and a subsequent cascade of events that ultimately results in synaptic potentiation (Todd et al. 2010). Additionally, tSCs respond with an oscillatory calcium response and a subsequent cascade of events that ultimately results in synaptic depression, in response to burst presynaptic nerve stimulation (Todd et al. 2010). tSCs release ATP, which is degraded into adenosine and then activates presynaptic A2a or A1 receptors (Todd et al. 2010). This study provides evidence for a model of balancing synaptic depression and

potentiation at the NMJ. tSCs have the ability, therefore, to decode presynaptic nerve activity and provide appropriate acute feedback relevant to current synaptic activity (Robitaille 1998; Todd et al. 2010).

tSCs also affect synaptic activity by modulating neurotransmitter release at the NMJ. One proposed hypothesis for the mechanisms underlying modulation of neurotransmitter release is tSC calcium response, or release of internal calcium stores from tSCs, which is necessary for release of an unidentified secondary messenger. Both release of tSC intracellular calcium and entry of extracellular calcium into the nerve terminal are required for neurotransmitter release modulation (Castonguay and Robitaille 2001). Release of glutamate from tSCs, activating metabotropic glutamate receptors (mGluRs) on the end-target muscle, is another proposed mechanism. In response to activation, the end-target muscle releases a chemical messenger that binds the presynaptic nerve, resulting in decreased presynaptic neurotransmitter release (Pinard et al. 2003). These studies reveal that tSCs are highly plastic, respond to synaptic activity, and participate in bi-directional modification: from synapse to tSC and tSC to synapse (Belair et al. 2010).

tSCs in NMJ Maintenance

tSCs have been demonstrated to be essential in maintaining the adult NMJ. A study conducted by Reddy and colleagues investigated the effects of tSC ablation at adult amphibian NMJs (Reddy et al. 2003). Ablation of tSCs was achieved via immune-mediated cell lysis. Adult frogs were injected locally at the end-target muscle with mAb 2A12, a monoclonal antibody that selectively recognizes the surface of tSCs. One day later, these muscles were injected with guinea pig complement serum, resulting in selective, complement-mediated cell lysis of tSCs (Reddy et al. 2003). Following acute tSC ablation, presynaptic nerve and muscle structure were unaltered, without evidence of nerve retraction or sprouting. Synaptic transmission was unchanged, with the frequency and amplitude of mepps (miniature end-plate potential), paired pulse facilitation, and synaptic depression showing no difference compared to pre-ablation, suggesting that tSCs may not acutely modulate synaptic transmission. These findings are contrary to the results published by Robitaille and Todd and colleagues discussed earlier in this review, that suggest that tSCs sense synaptic activity and provide timely, appropriate feedback to modulate synaptic transmission (Robitaille 1998; Todd et al. 2010). Recent studies suggest that tSCs have the ability to both potentiate and depress synaptic activity (Ko and Robitaille 2015). In the setting of tSC ablation, therefore, loss of both potentiation and depression could have a null effect on synaptic transmission in the acute setting (Ko and Robitaille 2015).

At 1-week post-tSC ablation, nerve terminals begin to retract, neurotransmitter release from the presynaptic nerve decreases, and muscle contraction deficits are present, suggesting that tSCs are essential for long-term stability and maintenance of the adult amphibian NMJ (Reddy et al. 2003). Additionally, studies have shown that through the extension and regression of tSC processes, tSCs are constantly remodeling nerve terminals in amphibians *in vivo* (Dickens et al. 2003; Macleod et al. 2001). Continuous remodeling in frogs is dissimilar to findings in mammals, where mammalian NMJs are remarkably stable and exhibit little remodeling (Lichtman et al. 1987; Wigston 1989). Regardless of the remodeling

differences between amphibian and mammalian NMJs, tSCs play a vital role in adult NMJ maintenance and remodeling.

tSCs and Peripheral Nerve Injury (degeneration and regeneration)

Peripheral nerve injuries are common, accounting for 5% of all trauma patients presenting to hospitals in North America (Noble et al. 1998). While over 50,000 peripheral nerve repair procedures are performed each year, (Evans 2001) the outcomes for many patients remain poor as only 10–40% of all patients ever regain baseline normal function (Portincasa et al. 2007; Scholz et al. 2009). Native muscle reinnervation following nerve injury is possible only during a critical window of time, approximately 12–18 months after nerve injury (Boyd et al. 2011; Lee and Wolfe 2012). Beyond this time interval, communication between the regenerating nerve and end-target muscle is no longer possible, resulting in permanent weakness, deformity, and paralysis. While the majority of the literature has focused on the site of nerve injury and speeding up neural regeneration, improved understanding of mechanisms that occur at the NMJ, downstream of the nerve injury, and more specifically at tSCs, is imperative to improve outcomes for patients affected by nerve injury.

tSCs are especially dynamic after acute nerve injury, or denervation. Beginning at three days after motor nerve injury, tSCs extend elaborate cytoplasmic processes, which serve as platforms for axonal growth (Kang et al. 2014; Kang et al. 2003; Reynolds and Woolf 1992; Son and Thompson 1995a; Son and Thompson 1995b; Woolf et al. 1992). These processes extend from denervated to innervated synaptic sites, forming “bridges” to allow intact, terminal nerves to more rapidly reach denervated synapses (FIGURES 4 and 5) (Kang et al. 2014; Kang et al. 2003). In addition to extending these processes that guide NMJ reinnervation, tSCs also acquire phagocytic activity which is necessary to facilitate successful nerve regeneration (Birks et al. 1960; Duregotti et al. 2015).

While tSC process extension after nerve injury has been described in several studies since 1992, (Kang et al. 2014; Kang et al. 2003; Reynolds and Woolf 1992; Son and Thompson 1995a; Son and Thompson 1995b; Woolf et al. 1992) our knowledge of the mechanisms driving tSC activation and tSC process extension beyond the area of the NMJ remain limited. The interaction between NRG1 and ErbB2 receptors, which are present on tSCs, may play a role in tSC process extension. The administration of exogenous NRG1 to neonatal muscles has been shown to activate ErbB2 receptors on tSCs, resulting in tSC process extension and the migration of tSCs away from endplates (Hayworth et al. 2006; Trachtenberg and Thompson 1997). Moreover, after denervation via α -latrotoxin, tSCs were activated by three mitochondrial alarmins: hydrogen peroxide, cytochrome c, and mitochondrial DNA. Additionally, inactivation of hydrogen peroxide delayed NMJ recovery after denervation, further suggesting the importance of this molecule on tSC activation and regeneration (Duregotti et al. 2015). A subsequent study found that the CXCL12 α -CXCR4 axis may also be important in NMJ regeneration after nerve injury. The authors show that tSCs produce CXCL12 α , and that blockade of this ligand delays NMJ recovery following acute nerve injury (Negro et al. 2017). Much remains to be learned regarding the mechanisms driving the activation and response of these cells after nerve injury. Future

studies are warranted to identify the molecular mechanisms that drive tSC process extension and to investigate the functional consequences of tSC loss.

Focus of Review

Given the significance of tSCs on formation, maintenance, remodeling, and reinnervation of the NMJ, it is evident why they have been implicated in neuromuscular diseases. This part of the review focuses on current evidence related to tSCs in the setting of neuromuscular diseases such as spinal muscular atrophy (SMA), Miller-Fisher syndrome, and amyotrophic lateral sclerosis (ALS), among others. While we cannot unequivocally imply etiological contributions of tSCs in these neuromuscular diseases due to the intimate interplay of structures within the NMJ, we emphasize that further tSC investigation may not only improve our understanding of disease pathogenesis, but may also help to identify innovative therapeutic strategies for the many patients who suffer from these neuromuscular disorders.

Spinal Muscular Atrophy

Spinal muscular atrophy is a recessive neuromuscular disease that is characterized by motoneuron loss in the spinal cord. It is a severe, progressive disease that primarily affects the proximal musculature. SMA affects approximately 1 in 6,000 to 1 in 10,000 live births, (D'Amico et al. 2011) making it the second most common autosomal recessive disease of childhood after cystic fibrosis. There are four types of SMA characterized by the age of onset and highest motor function that is achieved. Infants who suffer from SMA type I (Werdnig-Hoffman disease), for example, typically do not survive beyond age 2. Most patients do not ever acquire the ability to sit unsupported. In contrast, SMA type IV patients present during adulthood with a milder course as they are able walk and do not experience respiratory or nutritional difficulties (D'Amico et al. 2011). In humans, there are two *Survival Motor Neuron (SMN)* genes that can be affected in SMA patients: *SMN1* and *SMN2*. In 95% of patients with SMA, the *SMN1* gene is deleted. The disease results from having low functional levels of the *SMN* protein coded by the remaining *SMN2* gene. Moreover, small deletions or mutations of the *SMN1* gene account for the other 5% of patients with the disease (Lefebvre et al. 1995; McAndrew et al. 1997).

tSC Abnormalities in SMA Models

In severe SMA mouse models, transmission electron microscopy studies have determined that the diaphragm shows the earliest pathological changes. As early as postnatal day 4, diaphragmatic NMJs demonstrated variable amounts of disruption. Abnormal NMJs showed mitochondrial swellings in the axon terminals and muscle fibers. Moreover, tSCs displayed electron translucent, vacuole-like, cytoplasmic profiles (Voigt et al. 2010). A subsequent study performed by the same group found a difference in S100 (marker for tSCs) staining intensity between partially innervated endplates in SMA versus wildtype mice; reduced or absent S100 staining was observed in partially innervated, but not fully innervated, endplates. The authors hypothesized that this effect could be due to loss of SC identity, as indicated by the poorly stained tSCs, or displacement of the tSCs by different cells. They further postulated that these morphological changes in tSCs may affect the overall

maintenance and function of the corresponding NMJs (Neve et al. 2016). A different SMA model that displays a less severe disease phenotype demonstrates less NMJ remodeling after paralysis and progressive tSC loss, further supporting the tSC roles of NMJ maintenance and remodeling (Murray et al. 2013).

Miller-Fisher Syndrome

Miller-Fisher syndrome is a type of ascending peripheral neuropathy first described by Fisher in 1956. Known as a less common variant of Guillain-Barré, it is classically characterized by the triad of ophthalmoplegia, ataxia, and areflexia (Lo 2007). Most patients who are tested for Miller-Fisher syndrome possess serum positivity to anti-disialoside antibodies that bind GQ1b (i.e. anti-GQ1b IgG), and these antibodies were first described as a serological marker for Miller-Fisher syndrome in 1992 (Chiba et al. 1992). GQ1b is a ganglioside that has been isolated in the paranodal regions of cranial nerves (Chiba et al. 1997; Teener 2012). Gangliosides are also enriched in presynaptic membranes and at the NMJ (Halstead et al. 2004; O'Hanlon et al. 2001; Wirguin et al. 2002). While many cases of Miller-Fisher syndrome are self-limiting, intravenous immunoglobulins (IVIG) or plasmapheresis are the mainstays of treatment, if indicated.

tSCs and Miller-Fisher Syndrome

Evaluation of the anti-GQ1b antibody enhanced understanding of the pathophysiology of Miller-Fisher syndrome. A series of studies showed that GQ1b antibodies selectively target and damage presynaptic neuronal membranes and tSCs in animal models of Miller-Fisher syndrome (Halstead et al. 2005a; Halstead et al. 2008; Halstead et al. 2005b; Halstead et al. 2004; O'Hanlon et al. 2001; Overell and Willison 2005). These antibodies bind complement and induce a complement-dependent paralysis resembling α -latrotoxin-induced paralysis (Halstead et al. 2004; O'Hanlon et al. 2001). This group later identified that antibodies reactive with ganglioside GD3 together with complement induced tSC death, but had no effect on nerve terminal electrophysiology or morphology (Halstead et al. 2005b). Utilizing the methods described by Halstead and colleagues, (Halstead et al. 2005b) our group has confirmed specific tSC ablation in rodent NMJs *in vivo* without damage to the presynaptic nerve (FIGURE 3). These experimental studies demonstrate that complement-mediated tSC injury occurs in autoimmune neuropathy and suggest that tSC damage may result in human neuromuscular disease (Overell and Willison 2005).

Amyotrophic Lateral Sclerosis (ALS)

ALS is a progressive neurodegenerative disorder that affects 2 in every 100,000 adults. Due to a rapid loss in upper and lower motor neurons, patients suffer from muscle weakness and paralysis leading to death from respiratory arrest (Ilieva and Maragakis 2017). Although an abundance of literature has been dedicated to understanding the role of motoneuron loss in the central nervous system (CNS), significantly less is known about the degenerative mechanisms that occur distally at the NMJ, (Arbour et al. 2017) even though NMJ denervation precedes motor neuron loss and is a major determinant of disease severity and progression (Fischer et al. 2004; Gould et al. 2006). Because the NMJ is a complex structure

composed of several different cell types, understanding how each cell type is affected by and responds to ALS may shed light on disease mechanisms and help to identify new therapeutic approaches.

tSCs and ALS

One major cell type of investigative focus is the tSC given its location and ability to regulate, maintain, and repair the NMJ (Auld and Robitaille 2003; Feng and Ko 2008; Koirala et al. 2003; O'Malley et al. 1999; Robitaille et al. 1997). Several studies have implicated glial cells in the peripheral nervous system in ALS pathophysiology (Arbour et al. 2015; Carrasco et al. 2016a; Carrasco et al. 2010; Carrasco et al. 2016b; De Winter et al. 2006; Keller et al. 2009; Moloney et al. 2014). Using SOD1^{G37R} mice, which provide a model of slowly progressive ALS, Arbour and colleagues found that these animals had altered tSC ability to detect synaptic activities compared to wildtype controls prior to disease onset. Moreover, SOD1^{G37R} tSCs have enhanced intracellular Ca²⁺ changes associated with increased neurotransmitter release. This inappropriate tSC response may lead to improper NMJ repair and function during the disease course (Arbour et al. 2015). In addition to these functional abnormalities, Carrasco and colleagues demonstrated that SOD1^{G85R} and SOD1^{G93A} mice had morphological tSC abnormalities that were evident even before denervation occurred, suggesting further that tSCs may contribute to motor terminal degeneration and denervation in these ALS models (Carrasco et al. 2016b). A study performed by the same group demonstrated that tSC response after nerve injury was altered in mutant mice. Four days after nerve crush injury (i.e. denervation), tSCs at NMJs of SOD1 mice lost S100 labeling to a far greater extent than did the tSCs of wildtype, age-matched, control animals. Moreover, muscle reinnervation of SOD1 mice was delayed compared to wildtype animals, raising to question the ability of tSCs to support normal nerve terminal function in these SOD1 mice (Carrasco et al. 2016a).

De Winter and colleagues also showed that the axon repellent, Semaphorin3A (Sema3A), is differentially expressed in tSCs based on muscle fiber type. Specifically, Sema3A expression is increased in tSCs located on fast-fatigable muscle fibers. In the same study, the authors used G39A-hSOD1 transgenic mice, another mouse model for ALS, and showed a significantly higher number of NMJs with Sema3A-positive tSCs in pre-symptomatic mice versus in those with more progressive stages of the disease (De Winter et al. 2006).

In addition to tSC abnormalities in rodent ALS models, a study by Bruneteau and colleagues showed altered tSC morphology in human samples from nine ALS patients of differing disease severity (Bruneteau et al. 2015). tSC morphology was abnormal with extensive cytoplasmic processes on confocal microscopy. Using ultrastructural methods, some NMJs appeared normal while others demonstrated tSC processes abnormally invading the synaptic cleft, a finding that has been described in human samples from congenital myasthenic syndromes (see below). Given limited synaptic surface, tSC process intrusion into the synaptic cleft calls into question the ability for effective and efficient synaptic transmission in these abnormal appearing NMJs.

tSCs and other neuromuscular diseases

Abnormalities in tSCs have been found in other neuromuscular disease models. In *mdx* mice, the model for muscular dystrophy, tSCs located in the diaphragm extend processes, similar to those seen after denervation (Kang et al. 2003; Reynolds and Woolf 1992; Son and Thompson 1995a; Son and Thompson 1995b), but in a disorganized fashion and without bridge formation from denervated to innervated endplates (Personius and Sawyer 2005). Instead, these tSC processes are directed away from the endplates, possibly impairing the ability of dystrophic muscle to undergo reinnervation, resulting in muscle weakness and paralysis (Marques et al. 2006; Personius and Sawyer 2005). Insight into tSC abnormalities has been derived not only from rodent disease models, but also from human clinical samples. For example, samples obtained from patients with congenital myasthenic syndrome (CMS) caused by mutations in LAMB2 and agrin demonstrate invasion of tSC processes into the synaptic cleft or absent tSCs (Maselli et al. 2009; Nicole et al. 2014). While these neuromuscular diseases differ in presentation and prognosis, they share defects in tSCs, suggesting a potential role for tSCs in the pathophysiology of these disorders.

Conclusions

Despite the importance of tSCs on NMJ function and recovery, relatively little is known about these cells. tSCs are not only integral to synaptogenesis, but also to the health, maintenance, and plasticity of the synapse during homeostasis and after denervation. Here, we review studies demonstrating morphological abnormalities of tSCs present in multiple rodent disease models, such as SMA, ALS, muscular dystrophy, and autoantibodies (plus complement) that selectively target and lyse tSCs in Miller-Fisher syndrome. Additionally, human samples from patients with ALS and congenital myasthenic syndromes contain tSC alterations, which may contribute to disease phenotype. The tSC abnormalities noted to date in these pathological states are summarized in TABLE 1. tSCs are integral contributors to NMJ function and recovery, and show numerous pathological changes. An improved understanding of tSCs may help decipher the pathogenesis of disease and provide new therapeutic targets for the many patients who suffer from peripheral nerve injuries and neuromuscular diseases.

Acknowledgments

Grant information: Supported by the Plastic Surgery Foundation and the NIH National Institute of Neurological Disorders and Stroke Awards: F32NS098561 (to K.B.S.) and K08NS096232 (to A.K.S.W.).

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Significance Statement

Terminal Schwann cells (tSCs) are non-myelinating Schwann cells that reside at the neuromuscular junction (NMJ) and are important for synaptic development, maintenance, and remodeling. In addition to their roles in degeneration and regeneration after nerve injury, tSCs have been implicated in neuromuscular diseases, including spinal muscular atrophy (SMA), Miller-Fisher syndrome, and amyotrophic lateral sclerosis (ALS). An improved understanding of tSCs may lead to novel therapeutic strategies for these neuromuscular diseases and for nerve injuries.

Figure 1.

Main stages of Schwann cell (SC) development. All SCs are derived from the neural crest lineage. Myelinating and non-myelinating SCs (Pacinian corpuscles, Remak bundle, terminal SCs of NMJ) are derived from common precursors, immature SCs.

Figure 2.

Diagram (A) and confocal images (B, C) of the NMJ with nerve terminal (NT), non-myelinating terminal Schwann cells (tSC), post-synaptic acetylcholine receptors (AChR) and muscle fibers (M). tSCs (arrow, green) fluoresce under the S100b promoter in sternomastoid muscle of S100-GFP mice. **B.** Adult mice allow visualization of tSC bodies (arrow) **C.** At higher magnification, cytoplasmic processes are seen connecting tSCs within the NMJ (arrow). BTX= α -bungarotoxin (AChR, red); DAPI= nuclear staining (blue); Scale bar= 50 μ m (B) and 20 μ m (C).

Figure 3.

Immune-mediated tSC ablation in sternomastoid (SM) muscle of adult S100-GFP mouse *in vivo*. GD3 anti-disialosyl antibodies are specific for cell membrane glycosphingolipids in tSCs and lyse cells when topically applied to and injected into SM muscle in combination with complement (human serum). Ethidium homodimer-3 (EthD3, red) labels nucleic acids of membrane-compromised cells. **A.** Non-viable tSCs (arrow, red), ablated with 100µg of anti-GD3 antibody incubated for 30 min with human complement, are detected by EthD3 staining. **B.** Viable tSCs (arrow, green) are seen in control (Ctr) muscle. DAPI= nuclear staining (blue); Scale bar= 50µm.

Figure 4.

tSC response after nerve injury in mouse NMJs. **A.** Intact nerve and NMJs overlying muscle fibers (nerve fiber= green, myelin overlying nerve= red, tSCs= purple, muscle fibers= pink). **B.** After injury, Wallerian degeneration occurs, and tSCs begin to extend processes beyond the denervated NMJ. **C.** Long tSC processes form “bridges” with neighboring tSCs at innervated NMJs. **D.** Regenerating nerve fibers grow along tSC processes to speed NMJ reinnervation.

Figure 5. Extension of tSC cytoplasmic processes (arrows) after nerve injury in sternomastoid muscle of young adult S100-GFP mice. S100-GFP= tSCs and processes (green); BTX= α -bungarotoxin (red, B); DAPI= nuclear staining (blue); Scale bar= 20 μ m.

Table 1

Summary of tSC abnormalities in neuromuscular disorders. tSCs have been implicated in multiple neuromuscular disorders and rodent disease models. Specific tSC anomalies are listed by disease process.

tSC Anomaly	Pathological Process	Citations
Translucent, vacuole-like, cytoplasmic profiles (by electron microscopy)	SMA (rodent model)	Voigt et al. 2010
Reduced or absent S100 staining	SMA (rodent model)	Neve et al. 2016
Progressive tSC loss	SMA (rodent model)	Murray et al. 2013
tSC lysis	Miller-Fisher syndrome (rodent model)	Halstead et al. 2005
Altered detection of synaptic activities; enhanced intracellular Ca^{2+} release	ALS (rodent model)	Arbour et al. 2015
Extensive tSC processes beyond area of NMJ; tSC process invasion into synaptic cleft	ALS (human samples)	Bruneteau et al. 2015
Lack of tSC bridge formation	Muscular dystrophy (rodent model)	Personius and Sawyer 2005; Marques et al. 2006
tSC process invasion into synaptic cleft; absent tSCs	Congenital myasthenic syndrome (human samples)	Maselli et al. 2009; Nicole et al. 2014