Chapter

Non-Myelinating Schwann Cells in Health and Disease

Octavian Ioghen, Emilia Manole, Mihaela Gherghiceanu, Bogdan O. Popescu and Laura Cristina Ceafalan

Abstract

Non-myelinating Schwann cells (NMSCs) are one of the two major phenotypes of Schwann cells. NMSCs are of different types and have various locations. In the peripheral nervous system, NMSC, named Remak Schwann cells (RSC), accommodate multiple small-caliber axons, forming Remak bundles. NMSC, named perisynaptic/terminal Schwann cells, are found at the distal end of motor nerve terminals at the neuromuscular junction (NMJ). Thus, NMSCs proved to serve different functions according to their distribution such as maintenance of the axon and NMJ, peripheral nerve regeneration, or remodeling of the NMJ. Schwann cells (SCs) retain their proliferation capacity in the case of nerve injury or demyelination and provide support for the neuronal cells through paracrine signaling. Here we present an overview of their phenotypes and tissue distribution focusing on their emerging involvement in various peripheral nerve diseases.

Keywords: non-myelinating Schwann cells, Remak cells, perisynaptic Schwann cells, demyelination, nerve regeneration

1. Introduction

Among the Schwann cells (SCs), non-myelinating Schwann cells (NMSCs) represent an important category that was not extensively studied, although the gathered data demonstrate they are essential for axon maintenance and neuronal survival in the peripheral nervous system (PNS). Extending the knowledge on NMSCs biology could open new perspectives on the normal functioning of PNS as well as for better understanding the mechanisms underlying various pathological conditions and further on for developing new therapeutic approaches in peripheral nerve diseases.

The NMSCs encompass two major cell types, according to their distribution: Schwann cells of Remak fibers and the specialized perisynaptic/terminal Schwann cells at neuromuscular junctions (NMJ). In addition in this category are also included the glial cells found in some sensory transducers, such as the Pacinian and Meissner's corpuscles, as well as in the sensory and autonomic ganglia, where they are called satellite cells [1]. In pathological circumstances like axonal loss or demyelination, the former myelinating Schwann cells also become a class of NMSCs. Conversely, all NMSCs retain the potential to myelinate [2], if they receive the appropriate cues, most of which derive from the associated axons, along with some fate-controlling genes that act cell-autonomously within SCs [3, 4].

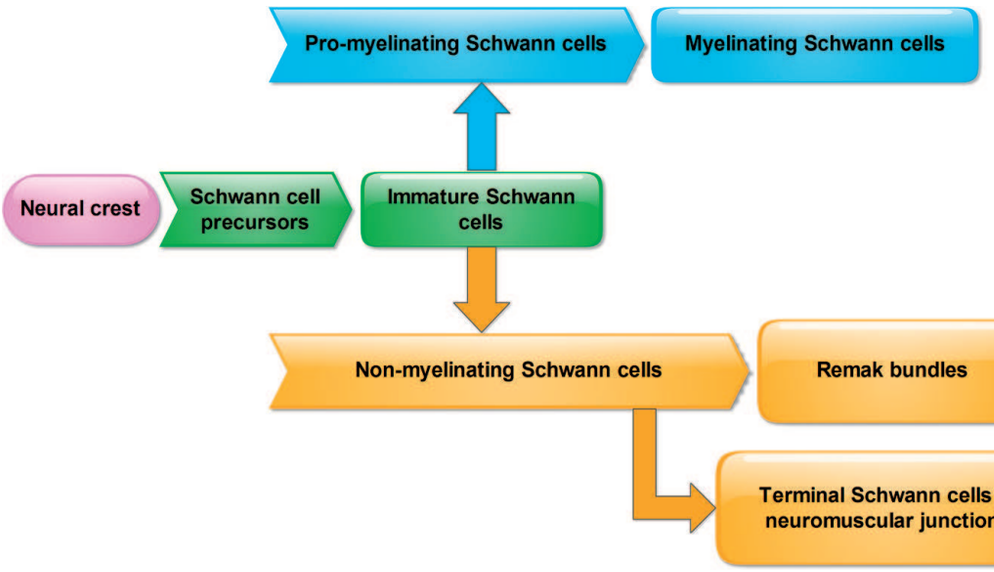

Figure 1.
Schwann cell lineage. SCs derive from the neural crest cells, after contacting nascent nerves during embryogenesis. Neural crest cells give rise to SC precursors, in early embryonic nerves which further differentiate into immature Schwann cells, in late embryonic and perinatal nerves. Postnatally, iSch will further differentiate either toward myelinating cells or non-myelinating cells according to axon-derived signals. The myelinating cells form the myelin sheath of large axons. The non-myelinating cells ensheath small axons forming unmyelinated fibers, called Remak bundles, or they migrate toward the neuromuscular junctions, covering the axon terminals, where they become terminal/perisynaptic/teloglia Schwann cells.

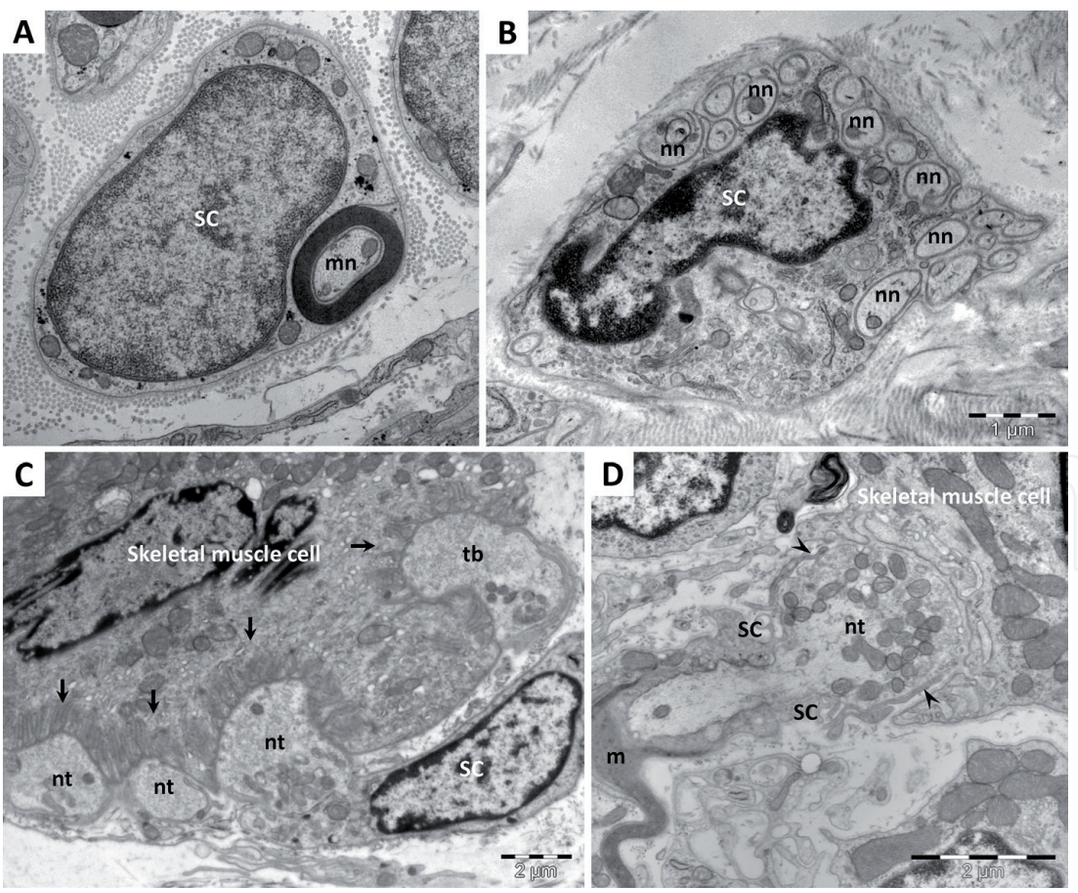

Figure 2.
Transmission electron microscopy of myelinated (mn, in A) and nonmyelinated (nn, in B) axons of peripheral nerves embedded in the cytoplasm of Schwann cell (Sch). C and D show the Schwann cells and nerve terminals (nt) in neuromuscular junction. (C) The motor end plate formed by folded sarcolemma (junctional folds, arrows) accommodates knob-like terminal buttons of the motor nerve (nt). (D) The myelin sheath (m) covering the axon ends (nt) in the vicinity of neuromuscular junction and Schwann cell extends into the synaptic cleft (arrowheads).

All Schwann cells derive from multipotent progenitor cells of the neural crest (**Figure 2**). The fate decision mechanism of SCs to become myelinating cells or to form RSCs is not fully understood, although the plasticity of SCs in various studies is recognized. Thus, some studies proved that if myelinated nerve segments are grafted, on a nerve that contains especially unmyelinated fibers, transplanted SCs do not myelinate, and equally, RSCs can produce a myelin sheath when they are grafted onto a myelinated nerve [2, 5].

After contacting nascent nerves during embryogenesis, neural crest cells give rise to SC precursors (SCP), which further differentiate into immature Schwann cells (iSC), in late embryonic and perinatal nerves (**Figure 1**). After birth, iSC will further differentiate either toward myelinating cells or non-myelinating cells according to axon-derived signals. The myelinating SCs form the myelin sheath of large axons (**Figure 2A**). The non-myelinating cells ensheath small axons forming unmyelinated fibers, called Remak bundles (**Figure 2B**), or they migrate toward the neuromuscular junctions, covering the axon terminals, where they become terminal/perisynaptic/teloglia SCs (**Figure 2C** and **D**).

This chapter addresses the main types of NMSCs, in terms of biological aspects and their role, aiming to highlight their importance for a better understanding of pathological mechanisms underlying various peripheral nervous system diseases.

2. Types of NMSC

2.1 Remak Schwann cells

Robert Remak first described the unmyelinated nerve fibers using the nerve fiber teasing technique in 1838 [6], so, in his honor, they were named "Remak fibers."

In the PNS most nerve fibers are unmyelinated [1], formed by RSCs accommodating a variable number of small-caliber axons (less than 1 μm diameter) (**Figure 2B**).

RSCs do not produce myelin, but they are essential for normal PNS development and functioning.

During PNS formation, pockets with multiple axons within a single mesaxon can be encountered. This aspect occurs only occasionally in normal adult Remak fibers where the small diameter axons of C nerve fibers (sensory/afferent), postganglionic sympathetic fibers, and some preganglionic sympathetic or parasympathetic fibers are accommodated in separate grooves of longitudinally interconnected RSCs forming the Remak bundles. Each RSC surrounds many axons, during radial sorting, forming a mesaxon for each axon. It is uncommon for an axon to be in direct contact with the basement membrane of the Schwann cell [4].

The number of axons surrounded by a RSC varies depending on the type of nerve fibers or a particular region along them. Thus, there is a higher number of axons exiting the dorsal root ganglion than in the distal segments of the peripheral nerve. In the cutaneous nerves, the number of axons per RSC decreases as they approach the skin [7], suggesting the existence of specific mechanisms regulating RSC-axons association as they approach their target. Moreover, the distribution of the axons within the Remak bundles varies along the peripheral nerve, with multiple axons within one pocket of the RSC toward the dorsal root and completely isolated axons in the distal segments [8].

There are studies reporting the presence of few short, myelinated internodes along a unmyelinated fiber especially in older animals [9].

Thus, it appears that the "ensheathment fate" of axons to either become myelinated or unmyelinated fibers relies on local/environmental cues. One of the most

extensively studied is the neuregulin 1 type III signaling through ErbB receptors, an axolemmal myelin-inducing factor [3] that promotes the formation of a mesaxon for each unmyelinated axon as well as SC differentiation into myelinating cells, depending on the expression level [10].

Another feature of unmyelinated nerve fibers is that axons may switch between neighboring Remak bundles along the nerve.

Moreover, a RSC can surround axons with different functions, for example, both sensitive and sympathetic axons, both axons expressing TrkA (tropomyosin receptor kinase A) receptors with a high affinity for nerve growth factor (NGF) and axons expressing RET (rearranged during transfection) receptors that respond to glial cell line-derived neurotrophic factor (GDNF) and artemin or axons derived from different dorsal ganglia [1].

2.1.1 Remak Schwann cell differentiation

The RSCs differentiation is governed, at least in part, by neuronal cues, especially by the signaling pathway neuregulin 1 type III (Nrg1-III)/ErbB2/ErbB3 receptor cascades. However, a number of cell-autonomous genes also contribute to SCs differentiation toward RSCs, one of which is gamma-aminobutyric acid type B1 receptor (GABBR1) [4].

SCs derive from the neural crest cells, after contacting nascent nerves during embryogenesis. Neural crest cells give rise to SCP, in early embryonic nerves, which further differentiate into iSCs, in late embryonic and perinatal nerves. Postnatally, iSCs will further differentiate either toward myelinating cells or non-myelinating cells according to axon-derived signals. The myelinating cells form the myelin sheath of large axons (larger than 1 μm diameter). The non-myelinating cells ensheath small axons forming unmyelinated fibers, called Remak bundles, or they migrate toward the neuromuscular junctions, where they become terminal/perisynaptic/teloglia Schwann cells (**Figure 3**).

2.1.1.1 Neuregulin

There are four distinct genes for neuregulins, but neuregulin 1 NRG1 is the best studied. NRG1, also known as glial growth factor (GGF), is a growth factor with EGF domain homology known to induce growth, differentiation, and migration of Schwann cells throughout development [10, 11]. NRG1 has three isoforms out of which type III is considered to be the most important signaling molecule for SC-axon interactions. NRG1 type III is produced by neurons and is released from axons by proteases, such as BACE1, or may remain anchored to the axonal membrane. NR1-III interacts with high-affinity tyrosine kinase receptors ErbB2/ErbB3 heterodimers, triggering the activation of downstream pathways, such as Ras/MAPK and PI3K/Akt SCs. Stimulation of mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) cascade was proven to lead to the suppression of myelinating state [12] through ErbB2 and ErbB3 receptors that are expressed in Schwann cells [13]. The NRG1-ErbB signaling pathway seems to play a crucial role in the SCs lineage for both myelinating and non-myelinating SCs and promotes SCP precursor survival after birth as well as during in vitro culturing [10, 14].

However, recent studies showed that in transgenic animal models where NRG1 is conditionally ablated during postnatal life, there is no reduction in the number of sensory axons but larger, unordered Remak bundles with polyaxonal pockets, where axons are not separated by SC processes, are formed, and some large-diameter

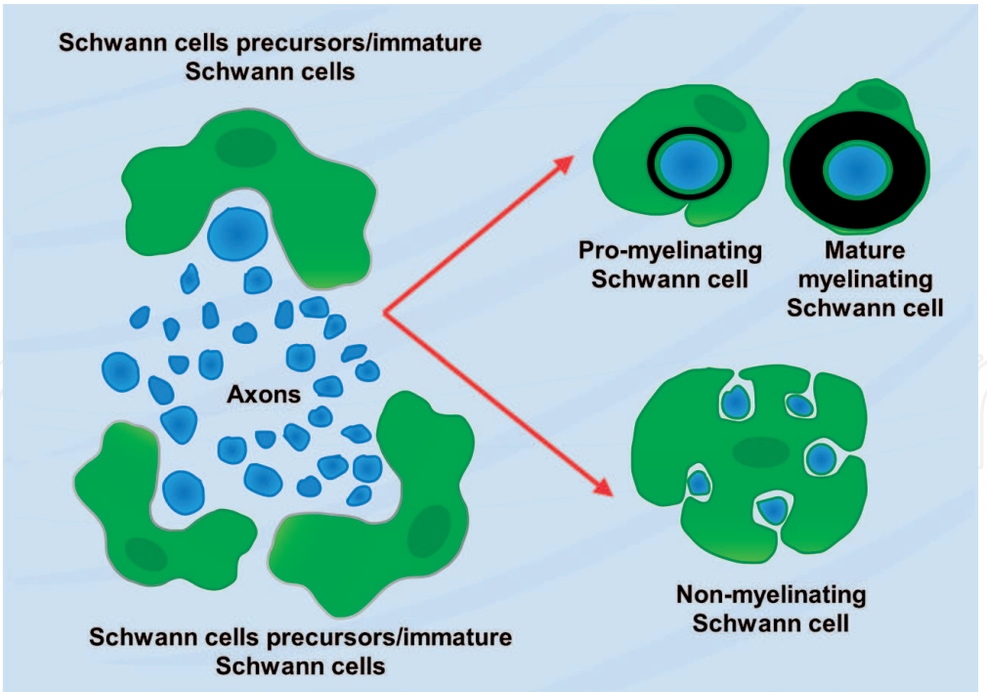

Figure 3.
Schwann cell development and maturation: their role in the evolution of myelinated and unmyelinated peripheral nerve fibers. Schwann cell precursors differentiate into immature Schwann cells which start the process of "radial sorting". A pro-myelinating Schwann cell envelops a large axon and becomes a myelinating Schwann cell. An immature Schwann cell which ensheaths many small axons becomes mature non-myelinating Schwann cell, forming a Remak bundle.

axons lose the myelin sheath. Only the sensory function was affected, without changing the survival and axonal maintenance of the neurons [15]. However, after nerve injury, RSCs re-establish normal Remak bundles, suggesting that during adulthood, after the basal lamina was established, axonal sorting is no more required [16].

Another experimental in vivo study on mouse sciatic nerve showed that NRG1 type III Erb2/Erb3 signaling regulates the morphological changes of the SCs. The study used a NRG1 type III knockout mouse model (+/−) with a low expression of NRG1 type III, which produced Remak bundles with a higher number of axons and smaller spaces between axons [17].

2.1.1.2 Genes acting cell-autonomously in Schwann cells

A number of studies have shown that there are certain genes that control SCs fate [4, 12, 18, 19] and that they act cell-autonomously in SCs. There are genes that can trigger upregulation of NRG1 during differentiation after injury, thus stimulating remyelination and redifferentiation of SCs [20].

An important genetically determining factor during SCs development is the gene for gamma-aminobutyric acid type B1 receptor (GABBR1), which is active mainly in RSCs as compared to myelinating SCs [21]. An in vivo experimental research showed that the absence of *GABBR1* in embryonic SCs leads to an increased number of small-caliber axons and Remak bundles and a decreased number of the large-caliber axons [19]. Furthermore, NRG1-III expression was decreased in GABBR1 mutant animals, in correlation with lower mean diameter axons along with a compensatory gene overexpression and protein levels of ErbB2 and ErbB3. Further studies are needed to analyze the requirement and the mechanism of these cell-autonomous genes in SC fate decision.

2.1.2 Remak Schwann cell maturation

During maturation, RSCs extend cell processes that individually encircle each axon with the plasmatic membrane and cytoplasm, separating it from surrounding axons. Naked axons, which were not completely surrounded by RSC cytoplasm and which come into direct contact with other axons, demonstrate failed RSC maturation after nerve injury [22]. Recent studies have shown that the expression level of a protein that is highly expressed in non-myelinating SCs, neuropathy target esterase (Nte), is correlated with SC developmental maturation and remyelination after neuronal injury. However, this protein is not involved in myelination [23].

Other proteins, such as mTOR [24–26], or G-G protein-coupled receptor Gpr126/Adgrg6, through laminin-211 and collagen type IV interactions, are required for both myelinating and non-myelinating SCs growth and function, during developmental stages as well as after nerve injury. Gpr126 controls radial sorting, myelination, SC-axon interactions, as well as Remak bundle formation [27–30].

In SCs, both deletion and overexpression of mTOR complex I adapter (Raptor) disrupts Remak bundle formation by increasing the number of axons in Remak bundles, with many naked axons [26], or decreasing the number of axons in Remak bundles and aberrant wrapping of multiple membrane-layered axons by RSCs, respectively [24, 31].

2.1.3 Role of Remak Schwann cells

The absence of myelin gives Remak fibers a certain plasticity, sprouting, and growth abilities that exceed that of myelinated fibers. That is why they are found especially in PNS, where the risk of physical injuries is much higher than in the CNS.

Although Remak fibers are found mainly in the PNS, they are also found in the CNS, associated with unmyelinated fibers in the parallel fiber system of the cerebellum and nigrostriatal pathway [1, 32].

2.1.3.1 RSCs as immune competent cells

NMSCs, like other SCs, can also function as immunocompetent cells playing an essential contribution in mounting and modulating of immune response in certain conditions, by antigen presentation and cytokine secretion, as well as by their direct interaction with immune cells. Moreover, NMSCs express specific pattern recognition receptors (PRR) for the detection of pathogens, such as Toll-like receptors (TLRs) and the nucleotide-binding and oligomerization domain (NOD)-like receptor (NLR) family [33–35].

The crosstalk between immune- and peripheral nerve SCs through a large array of molecules either expressed or recognized by SCs build up the base for nervous-immune system interactions. The subject was extensively reviewed by Tzekova et al. [34]. Moreover, Hu et al. showed that NMSCs located in the thymus develop correlations with thymocytes, lymphocytes, and dendritic cells under normal and pathological conditions. They concluded that NMSCs are highly suitable for studying the local interactions of the PNS and primary lymphoid tissues or organs [36]. The same observations were made by Ma et al. studying the mouse spleen and the interactions between NMSCs and leukocytes [37].

Another role for NMSCs was concluded by the study of Yamazaki et al. which showed that NMSCs maintain hematopoietic stem cell hibernation in the bone marrow niche. They demonstrated that NMSCs proved responsible for activation of TGF-beta latent form. These glial cells, ensheathing autonomic nerves, get in

contact with hematopoietic stem cells and maintain them in hibernation by regulating activation of latent TGF-beta [38].

2.1.3.2 RSCs in nerve injury and regeneration

Transection of a nerve fiber initiates Wallerian degeneration of the distal stump. As opposed to oligodendrocytes, SCs maintain the ability to dedifferentiate to an immature phenotype in response to nerve injury or disease, and they can actively promote the repair and functional recovery. The repair SCs express inflammatory mediators, such as interleukins and TNFα, as well as anti-inflammatory cytokines (IL-10, Epo, or TGFβ) and growth factors shown to promote Wallerian degeneration, macrophage attraction, and axonal regeneration upon nerve injury [34].

A number of molecules have been shown to play important roles in modulating SC behavior after nerve injury.

LDL receptor-related protein 1 (LRP1) is a significant factor involved in the development and maintenance of Schwann Cells, both myelinating and NMSCs [39]. LRP1 is one of the molecules upregulated after various types of peripheral nerve injury.

The study of Campana et al. proved that LPR1 upregulation was directly correlated with local production of TNFα and TNFα/LPR1 signaling is one of the survival mechanisms for SC migration and survival observed in in vitro studies [40].

Another signaling receptor that plays an important role in regulation of Schwann cell-axon interactions is fibroblast growth factor receptor (FGFR). Fibroblast growth factor 2 (FGF2) is one of the essential regulators of peripheral nerve regeneration after injury [41]. Three of its receptors, expressed by Schwann cells and dorsal root ganglia neurons, are FGFR1, FGFR2, and FGFR3 which are all upregulated after nerve injury [42].

One day after nerve transection, all SCs start to proliferate within the basal lamina. One week post-injury, RSCs double in length, and after 4 weeks they are three-fold longer and were called repair-supportive Schwann cells. About 50% of repair cells derive from RSCs. The loss of axonal contact determines cells to branch. They form branches lying parallel to the main cell axis, building cellular columns and Bungner bands distal to injury site and offering the support of regenerating sprouts. They will further differentiate to myelinating cells after regeneration [43].

2.1.3.3 RSCs and sensory nerve fiber pathology

Most unmyelinated C-fibers ensheathed by Remak cells are nociceptors [39]. They transmit pain information to the brain. Thus, the dysfunction of RSC induces an altered transmission of the nociceptive stimuli, which leads to severe neuropathic pain.

The specific loss of GABBR1 in SCs results in an increased number of C-unmyelinated fibers, leading to a hypersensitivity to thermal and mechanical stimuli. There is also an alteration of the locomotor coordination, without any injury. It is not known whether these consequences are caused only by the modification of the unmyelinated axon number [19].

Other in vivo studies showed that after injury, in LRP1 knockout animals, the resulting hypomyelination and impaired RSCs ensheathment lead to motor dysfunction and mechanical allodynia [39] without any traumatic injury. These pathological changes can cause notable painful symptoms such as mechanical allodynia [39]. In a model with partial nerve injury, the LRP-negative mice have a higher degree of RSC apoptosis, an accelerated degeneration, and further more severe pain in the LRP than the nonmutant mice [39]. These findings suggest the involvement

of RSC in the pathophysiology of neuropathic pain and the importance of LRP1 in the physiology of RSC and open the possibility of using RSC as a new therapeutic target in the treatment of neuropathic pain.

In an experimental study in vivo on FGFR1 and FGFR2 single and FGFR1/FGFR2 double conditional knockout mice, Furusho et al. showed that lack of FGFR1 and FGFR2 signaling in NMSCs resulted in sensory axonal neuropathy in unmyelinated C-fibers and the impairment of thermal pain sensitivity [42]. Another study by Chen et al. performed on transgenic mice that postnatally express a dominant-negative ErbB receptor in NMSCs but not in the myelinating ones led to a progressive peripheral neuropathy with loss of unmyelinated axons and heat/cold pain [44]. Altogether, such data suggest the important role of RSCs in in the modulation of pain sensitivity in peripheral sensory neuropathies.

Charcot-Marie-Tooth type 1A (CMTA1A) is a genetic disease of the peripheral nervous system in which demyelination and further aberrant remyelination occur in a repeated cycle, with an "onion bulb" appearance in microscopy. From the clinical point of view, CMT1A is characterized by weakness and muscle atrophy in the lower limbs and later on by sensory loss. Myelinating Schwann cells are classically known to be impaired in CMT1A, but it seems that there is also an impairment of the RSC [45]. A proliferation of RSC takes place as a response to the degeneration of the myelinated axons that appear to secrete mitogenic factors [45]. Unexpectedly, no degeneration occurs in the unmyelinated fibers [45]. These findings reveal that RSC are altered in CMT1A, but without any impact on the unmyelinated fibers, in comparison to the relation between myelinating SCs alteration and degenerated myelinated axons. Further studies need to elucidate the contribution of RSC to the pathogenesis of CMT1A.

2.2 Perisynaptic (terminal) Schwann cells (PSCs)

2.2.1 PSC phenotype and distribution

PSC, also known as teloglia or terminal Schwann cell, is a type of non-myelinating Schwann cell which is found above the presynaptic nerve terminal at the level of the NMJ. Louis-Antoine Ranvier described in 1878 the presence of a type of cell in the NMJ distinct from the axon terminal or the muscle fiber. He named this cells "arborization nuclei" because of their widespread projections along the NMJs. Later on, with improved histology techniques and in the era of electron microscopy, several studies identified the presence of this specific type of cell in the NMJ (**Figure 2C**).

PSCs express several markers that are used to highlight them in situ. The most common approach used is anti-S100b immunolabeling [46]. S100b is a nonspecific marker for all types of SCs, either myelinating or non-myelinating ones. In amphibians only, to distinguish PSCs from myelinating SCs, two specific antibodies are used, peanut agglutinin (PNA) [47] and 2A12 monoclonal antibody [48], which mark the extracellular matrix and the cells' surface, respectively. Interestingly, PSCs express several myelin proteins such as myelin-associated glycoprotein (MAG), galactocerebroside, protein zero (P0), and 2′,3′-cyclic nucleotide 3′-phosphodiesterase [49]. The cells are not involved in the process of myelination, though the presence of these proteins proves the common origin of the two types of Scs. Additionally, PSCs express on their surface several receptors such as acetylcholine receptors, ATP, purinergic receptors, and L-type voltage-dependent calcium channels that usually take part in the synaptic transmission [50–53] supporting the hypothesis that PSCs play an active role in the NMJ rather than having only a structural role.

Several studies determined that the number of PSCs gradually increase after birth [54]. Adult NMJ may contain one up to five PSCs [55–57], and their number is modulated by PSC-muscle cross talk through neurotrophins [58].

PSCs tend to be positioned at the presynaptic side, on top of the motor axon terminal, without the intervention of a basal lamina [55, 56]. Recently a new population of fibroblast-like cells named kranocytes—NMJ-capping cells—was detected on the other side, above the basal lamina of the PSC, covering all other cells of the NMJ. They are thought to have important roles in the NMJ repair after nerve injury [59, 60]. Kranocytes appear to communicate with PSCs via neuregulin signaling pathway to act synergistically after nerve damage [59].

Most studies about PSCs were performed either on amphibian (frog) or rodent (mouse) samples [53]. A peculiarity of the frog's NMJ, where the unmyelinated nerve terminal is completely surrounded by PSCs and does not form dilated terminal buttons and the synaptic contact is formed all along, is that PSCs send finger-like projections into the synaptic cleft, on the presynaptic side, which separate, at a regular distance, active areas where the neurotransmitters are released from covered areas [52, 61]. These active areas correspond on the opposite side to the folds of the sarcolemma, the postsynaptic element of the NMJ, which are rich in nicotinic acetylcholine receptors [52, 61]. In mammals, PSC projections do not reach the synaptic cleft (**Figure 2D**).

2.2.2 PSC roles in the formation and function of the neuromuscular junction

PSCs are involved in the growth and maintenance of the NMJ during development.

Although these cells do not take part in the initial formation of the axon-muscle junction, PSCs have key contributions in the next stages of NMJ development. In animal models lacking SCs, the axon reaches the muscle in the initial step of the NMJ formation, but only for a brief time [62, 63]. In the absence of SCs, the NMJ gets disrupted, suggesting the vital role of PSCs in the NMJ maintenance during development [64]. Soon after the contact between the axon and the muscle, PSCs intensively divide, sprout, and are primarily involved in the growth of the synapse [64].

PSCs are also involved in the physiological processes of polyneuronal innervation and synapse elimination. PSCs are involved in the multiple innervation process of the muscles and suffer a regression in parallel with the axonal withdrawal [1, 65, 66]. After the process of axonal withdrawal, PSCs are engaged in the removal of nerve debris, through phagocytosis [67].

The signaling pathway which facilitates the survival and growth of PSCs and the tight communication between PSCs and motor axons is the neuregulin1-ErbB pathway [1].

PSCs have important roles in the maintenance of the NMJ during the adult life as the structural support. Ablation in PSCs on the adult NMJ does not impede the immediate structure and function of the synapse, but after a period of time, the motor axon terminals retract, and the NMJ collapses [64, 68]. Thus PSCs have a significant contribution to the structural maintenance of the synapse under the action of physical factors such as the intense tractions between the nerve and the muscle [53].

These cells dynamically participate in the process of synaptic transmission of information between the motor axons and the muscles, having an important role in the modulation of NMJ activity [53, 57, 69]. Not only PSCs can alter the synaptic transmission, but PSC activity can also be modified by synaptic transmission. Or, as some authors like to say, PSCs can both "talk" and "listen" in the synapse [53, 69].

When the nerve terminal increases its firing rate and a large amount of neurotransmitter is released in the synaptic cleft, a simultaneous increase of intracellular calcium occurs in PSCs [70, 71]. A similar effect is obtained by applying exogenous acetylcholine and ATP, molecules normally released by the synaptic vesicles, to PSCs [51]. Moreover, the levels of intracellular calcium vary depending on the type of the nerve firing rate, either burst or continuous [72]. These events do not occur in the myelinating SCs and emphasize the detection of synaptic activity by PSCs and the modification of their cellular behavior secondary to the synaptic transmission [69]. This is similar to a decoding process of the synaptic activity. Thereby, the events correspond to the "listening" ability of PSCs in the synapse.

The increase of the PSC intracellular calcium levels does not play only a "decoder" role. This transient raising modulates the synapse by intensifying the neuromuscular transmission. PSCs are expressed on the surface of several G protein-coupled receptors with contributions in the modulation of the synapse activity [73]. Evidences suggest that different ligands of these G protein-coupled receptors determine different changes in the neuromuscular transmission, as follows: a GTP analogue decreased the neurotransmitter release, while a GDP analogue reduced the synaptic depression [73]. These events correspond to the "talking" ability of PSCs in the synapse.

Therefore, PSCs are not only a structural, passive component of the NMJ, but an active one. These evidences confirm that the NMJ is a tripartite synapse.

2.2.3 Roles in pathology

PSCs induce and guide the growth of nerve sprouts to re-establish the NMJ after nerve injury.

All the actions that PSCs perform in an attempt to regain the activity of the NMJ appear to be mediated by neuregulin1-ErbB signaling pathway [74].

First of all, after nerve degeneration, PSCs develop phagocytic traits for the clearance of the debris from the nerve terminals [75].

Second of all, PSCs are involved in the guiding of reinnervation. A few days after the nerve injury, PSCs from the altered NMJ begin to abundantly sprout, and these new processes reach adjacent undamaged synapses [76]. In this manner, "bridges" are established between the innervated and the dennervated NMJs. The role of the newly formed bridges is to facilitate the nerve pathway to find the altered NMJ and to regenerate the synapse more rapidly [69, 76]. However, satellite NMSCs seem to play a role in nerve regeneration after insult as well and might be involved in pathogenic pathways of neuropathic pain [77].

Miller Fisher syndrome is a Guillain-Barré syndrome variant with antibodies against GQ1b ganglioside that is clinically characterized by ataxia, ophthalmoplegia, and areflexia. Studies on mouse models revealed that PSCs represent an important target of the autoimmune process, the cellular destruction is complement dependent, and this pathogenic mechanism might be relevant for the human disease [68, 78].

Amyotrophic lateral sclerosis (ALS) is a challenge for both the clinician and the researcher due to the obscure pathological mechanisms that are still not completely understood. The role of glial cells in the pathophysiology of the disease is not clear yet. Most probably the SC modifications are a consequence of the neurodegeneration process. However in human patients with ALS, PSCs have abnormal features with cellular processes that extend into the synaptic cleft [79]. Additionally, in ALS mouse models, PSCs have abnormal intracellular levels of calcium, causing a flaw in the synaptic "decoding" function [80].

Another neuromuscular disease in which PSCs appear to be involved is spinal muscular atrophy (SMA). In an ultrastructural study on SMA mouse models, PSCs in the diaphragmatic muscle show changes in their morphology such as vacuole-like translucent profiles and an electron-dense cytoplasm [81]. Another study on SMA mouse models revealed that in the evolution of the disease, there is a progressive loss of PSCs, leading to an improperly remodeling and regeneration of the NMJ [82].

3. Conclusions

Although little is known on the NMSC, they are very important players for normal PNS function. Recent studies showed that RSCs play a very important role in the development of peripheral nerves and regeneration after injury. RSCs are also involved in the modulation of pain sensitivity in peripheral sensory neuropathies. Even in the absence of injury, disturbance in axonal-RSC interaction is followed by neuropathic pain.

Additionally, PSCs are mandatorily involved not only in synaptogenesis but also in the growth and maintenance of the normal synapse as well as after denervation. Morphological changes of PSCs were detected in various pathological conditions suggesting their potential involvement in the pathogenic mechanism of such diseases.

A better understanding of the molecular mechanisms that govern the development and functioning of NMSCs could broaden the perspective on the pathogenesis and potential therapeutic targets for neuropathy and peripheral nerve injuries.

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Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

SCs	Schwann cells
RSC	Remak Schwann cells
NMSCs	nonmyelinated Schwann cells
NGF	nerve growth factor
GGF	glial growth factor
ERK	extracellular signal-regulated kinase
Nte	neuropathy target esterase
FGFR	fibroblast growth factor receptor
NRG1	neuregulin 1
Nrg1-III	neuregulin 1 type III
GDNF	glial cell line-derived neurotrophic factor
GABBR1	gamma-aminobutyric acid type B1 receptor
SCP	SC precursors
iSch	immature Schwann cells

PSCs	perisynaptic Schwann cells
NMJ	neuromuscular junction
PNA	peanut agglutinin
ALS	amyotrophic lateral sclerosis
CMT1A	Charcot-Marie-Tooth type 1A

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