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RESEARCH ARTICLE

Satellite glial cells represent a population of developmentally arrested Schwann cells

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Abstract

Satellite glial cells (SGCs) are glial cells in the peripheral nervous system that form sheaths around the neuronal cell body. This unique arrangement of SGCs allows it to exert a highly regulated control over the neuronal microenvironment. Not much is known about the origin of SGCs. In this study, we examine the development of SGCs. We show that rat SGCs develop postnatally and these cells express a number of markers associated with Schwann cell precursors, in particular cadherin-19 (CDH19) even in adult DRGs. We developed a method for the purification of SGCs and showed that they are transcriptionally and morphologically very similar to adult rat Schwann cells (SCs). Finally, we demonstrate that purified SGCs are capable of myelinating embryonic axons when cocultured with those axons. Based on these observations we hypothesize that SGCs represent a population of cells in the SC lineage, whose further differentiation appears to be arrested through contact with DRG neuronal soma.

KEYWORDS

cadherin-19, myelin, satellite glial cells, Schwann cells

1 | INTRODUCTION

Primary afferent neurons transmit peripheral information to the central nervous system (CNS) and their soma is localized within the trigeminal and the dorsal root ganglia (DRG). There, these soma are surrounded by sheaths of cells known as satellite glial cells (SGCs; Pannese, 1981, 2010). SGCs have been ascribed to have functions similar to those of astrocytes in the CNS, in that they control the microenvironment around the neural soma and hence can modulate the activity of these neurons through complex SGC, neuronal soma cross communication (reviewed in Costa & Moreira Neto, 2015; Huang, Gu, & Chen, 2013). In addition, they exhibit an 'activated' state in response to neural injury, and propagate that state to other SGCs through calcium signaling (Donegan, Kernisant, Cua, Jasmin, & Ohara, 2013; Nadeau, Wilson-Gerwing, & Verge, 2014; Suadicani et al., 2010). This phenomenon results in the recruitment of other neurons to the injury response, hence, implicating SGCs in the development of chronic pain (Dublin & Hanani, 2007; Suadicani et al., 2010). In addition to their role in neuronal soma signaling, SGCs have also been proposed to be highly plastic, giving rise to a range of cells that includes oligodendrocytes (Fex Svenningsen, Colman, & Pedraza, 2004; Hanani, Huang, Cherkas, Ledda, & Pannese, 2002; Weider et al., 2015). SGCs have also been shown to undergo enhanced proliferation in response to nerve injury (Humbertson, Zimmermann, & Leedy, 1969) and have been hypothesized to generate new neurons within the DRG after neuronal loss as a result of injury.

Although derived from the neural crest (Jessen & Mirsky, 2005), the lineage of SGCs remains relatively uncharacterized. Their plastic nature and the fact that elevated levels of the sox10 transcription factor promote the conversion of SGCs into an oligodendrocyte type cell, suggest that SGCs may not represent a fully differentiated cell type (Weider et al., 2015), an idea supported by the expression of the transcription factor sox2, which has been described as a stem cell marker, in the nuclei of SGCs (Koike, Wakabayashi, Mori, Hirahara, & Yamada, 2015). The lack of knowledge concerning the development of SGCs within the trigeminal ganglia or DRG contrasts what is known about the development of the other PNS glial cell type, the Schwann cell (SC), which has been well characterized in developing nerves such as the sciatic. In the early sciatic nerve, neural crest cells undergo three different developmental stages to differentiate to a mature myelinating or non-myelinating SC. Each of these stages are defined by a set of markers they express, including unique markers such as cadherin-19 (CDH19), which is solely expressed during the SC precursor stage (Takahashi & Osumi, 2005). The SC precursors are transient and are seen during

embryonic day 14 and are dependent on axonal contact for survival. Around embryonic day 15–17, they develop autocrine survival signals and differentiate into immature SCs. The cells then differentiate to a mature myelinating or non-myelinating SC during perinatal stages (reviewed in Jessen & Mirsky, 1997; Mirsky et al., 2008). Whether SGCs have similar developmental stages or signals that leads to their specification has not been investigated. However, certain clues suggest that SGCs may be closely related to SCs. In sox10 mutants, both SGCs and SC precursors fail to develop, suggesting the potential for a common lineage (Britsch et al., 2001). Moreover, when cultured independently of neurons, SGCs start to adopt the characteristic bipolar morphology associated with SC (Belzer, Shraer, & Hanani, 2010; Poulsen, Larsen, Duroux, & Gazerani, 2014) and express several biomarkers (e.g., GFAP, S100, and L1) also observed in SC.

In this study, we provide data to support the hypothesis that SGCs in the DRG represent a population of cells arrested in the SC lineage progression. We find that SGCs do not associate with neuronal soma until after birth and the SC precursor marker CDH19 along with other markers of the early SC lineage are then expressed through adulthood. Using a novel purification technique we observed that purified cultures of SGCs are almost transcriptionally identical to cultures of adult SCs. Finally, we showed that coculturing SGCs with pure populations of embryonic DRG neurons allowed them to myelinate DRG axons in the same manner as adult-derived SC. These observations raise new questions about the DRG microenvironment as a stem cell niche and the hierarchy of glial cell development in the PNS.

2 | MATERIALS AND METHODS

2.1 | Animal use

Cervical DRGs were isolated from E15, P2, and P90 female Sprague Dawley rats for the study. The animals used were approved by Institutional Animal Care and Use Committee.

2.2 | Generation of adult rat SCs

Sciatic nerve from 90-day-old rats were isolated under aseptic conditions and SCs were generated as described previously (Morrissey, Kleitman, & Bunge, 1991) with some modification. Briefly, the sciatic nerve was cut into 1 mm long segments and maintained on uncoated 24-well plates (Fisher, Hampton, NH). The cultures were fed twice a week with DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% HIFBS (Hyclone Laboratories, Logan, UT) to facilitate fibroblast migration from the nerve. After formation of a confluent layer of fibroblasts, the segments were transferred to a new uncoated 24-well plate. This was repeated about 3–7 times till the outgrowth appeared to be primarily SCs based on morphology. The segments were then incubated overnight at 37°C in 0.05% stemzyme (Worthington Biochemical Corp, Lakewood, NJ), 25 mM HEPES (Sigma, St. Louis, MO) buffer supplemented with 15% FBS and 1% penicillin/streptomycin (P/S) in DMEM. The following day, the segments were dissociated by trituration with a fire polished glass pipette. The cell suspension was centrifuged at 235*g* for 5 min. The pellet was then resuspended in D10M (DMEM supplemented with 10% HIFBS, 2 μM forskolin (Sigma), 20 μg/ml bovine pituitary extract (Biomedical technologies Inc., Stoughton, MA) and 1% P/S) media. SCs were isolated by laminin selection as previously described (Pannunzio et al., 2005) with some modifications. Briefly, SCs were plated on coverslips coated with 50 μg/ml Poly-L-Lysine (Sigma) and 10 μg/ml laminin (Invitrogen). After initial seeding for 10 min, the media from the coverslip containing the cells (well 1) was transferred to a similarly coated coverslip (well 2) and fresh media was added to well 1. Laminin selection was repeated three to four times to effectively remove myelin debris.

2.3 | Isolation of SGCs

Cervical DRGs from female Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were isolated from 90-day-old animals (P90, adult DRG; Malin, Davis, & Molliver, 2007; Sleigh, Weir, & Schiavo, 2016). The epineurium and nerve roots were removed and the DRGs were transferred to 5 mg/ml collagenase type 2 (Worthington). The isolated DRGs were incubated at 37°C for about an hour with agitation every 15 min. The ganglia were then triturated using a fire polished glass pipette until the tissue was completely homogenized. The solution was then spun at 1,500 rpm for 5 min and the collagenase was removed. The pellet was resuspended in about 2 ml of NB media (neurobasal media [Invitrogen], 2% B27 [Invitrogen], 1% GlutaMax [Invitrogen], 1% P/S [Invitrogen] and 50 ng/ml NGF [Harlan Laboratories, Indianapolis, IN]) and was extensively triturated. This cell suspension was then carefully layered on a cushion of 10% and 5% BSA (Amresco, Solon, OH). The cushion with cells was centrifuged at 115*g* for 4 min (low brake). The SCs and the myelin debris were observed at the interface of the cushion and the neurons with associated SGCs pelleted. The supernatant was discarded and the BSA cushion separation was repeated again to remove any remaining contaminating SCs and debris. The pellet from this stage of the preparation is referred to as fraction 1. To obtain purified cultures of SGCs and DRG neurons, fraction 1 was further treated with 0.25% trypsin solution (Life Technologies, Carlsbad, CA) at 37°C for about 45 min. At the end of the incubation, the cells were triturated and centrifuged at 1,500 rpm. Trypsin was removed and the cells were resuspended in seeding media and layered again on a BSA cushion to perform the separation. SGCs which have now been removed from the neurons settle at the interface of the BSA cushion while pure neurons pellet. The pellet containing neurons is referred to as fraction 2 and the supernatant containing SGCs is referred to as fraction 3. Isolated SGCs (fraction 3) were plated on 12 mm German glass coverslips (Carolina Biological, Burlington, NC) or dishes (Sigma) coated with 200 μg/ml Poly-L-Lysine (Sigma). The neurons (fraction 1 and fraction 2) were grown on coverslips coated with 0.01% poly-L-ornithine (Sigma) and 50 μg/ml laminin (Invitrogen). The cultures were fed every 2–3 days with NB media as mentioned above. Data ($n = 3$) was analyzed by ANOVA followed by Bonferonni's post hoc test.

2.4 | Preparation of dissociated embryonic DRG neurons, neuron/SC and neuron/SGC cocultures

Embryonic day 15 (E15) DRGs were isolated and dissociated as described previously (Fex Svenningsen, Shan, Colman, & Pedraza, 2003; Hall, 2006). Dissociated DRGs were seeded on laminin (50 μg/ml) coated 12 mm German glass coverslips at a density of one DRG per coverslip. The cultures were fixed and analyzed at 1, 5, and at 12 days. For endogenous myelination of embryonic DRGs, the cultures were grown for 7 days and then switched to myelin permissive media (NB media with 50 μg/ml L-Ascorbic acid) for 14 days (21DIV; Callizot, Combes, Steinschneider, & Poindron, 2011).

For coculture experiments, dissociated embryonic DRGs were treated with one three day pulse of 10 μM FUdR (Wood, 1976). Pure neurons were seeded with either SGCs or SCs 7 days following the removal of FUdR (12DIV) at a density of 100,000 cells per coverslip. Since the SCs were grown in 10% HIFBS containing media, we gradually decreased the serum concentration in the media over a period of 7 days and switched to serum free NB media. At 19DIV, the cocultures were switched to myelin permissive media. The cultures were maintained for another 21 days in myelin permissive media after which the cultures were fixed for analysis (40DIV). Myelin segments were quantified ($n = 3$) using Volocity 6.3 software.

2.5 | Endogenous myelination of adult DRGs

Cervical DRGs from 90-day-old rats were isolated as mentioned above and individual DRGs were placed on coverslips coated with 0.01% poly-L-ornithine and 50 μg/ml laminin. For endogenous myelination of dissociated adult DRGs, fraction 1 was obtained as mentioned above. Both explant and dissociated culture were allowed to grow for 14 days in NB media. In the explant culture, the volume of media was kept low (about 35 μl maintained as a bubble on a 12 mm coverslip) to facilitate attachment. At 14DIV, they were switched to myelin permissive media and were maintained for another 21 days (35DIV).

2.6 | Immunohistochemistry and immunocytochemistry

Cervical DRGs were harvested from 15-day-old embryos (E15), 2 day (P2), and 90 day (P90) old postnatal rats. DRGs were fixed with 4% paraformaldehyde (PFA) in PBS for 30 min, rinsed and then transferred to 30% sucrose with 0.02% sodium azide solution overnight at 4°C. The tissues were embedded in Tissue Tek OCT compound (Sakura, Torrance, CA) and 10 μm thick sections were placed onto positive charged microscopic slides (Medline Industries, Mundelein, IL). For *in vitro* studies, the cells were fixed in 4% PFA for 10 min, followed by permeabilization with 0.3% triton X-100 in 4% PFA for 10 min. The cultures were then washed with PBS and blocked for 30 min in 10% normal goat serum (myelinating cultures were incubated in 10% normal goat serum containing 0.1% triton X-100) and incubated overnight at 4°C with primary antibodies mouse TUBB3 (1:300, abcam, Cambridge, MA), rabbit GS (1:10,000, Sigma), rabbit MBP (1:200, abcam), rabbit Ki67 (1:50, abcam), rabbit CDH19 (1:100, Santa Cruz Biotechnology, Dallas, TX), rabbit S100 (1:200, Dako, Carpinteria, CA). The cultures were then incubated for 45 min at room temperature with secondary antibodies (goat, donkey-Alexa Fluor; 1:1000; Invitrogen) and DAPI (Invitrogen) and were mounted in Fluorogel (Electron Microscopy Sciences, Hatfield, PA). Images were acquired with Zeiss LSM710 confocal microscope and ZEN 2.1 software.

2.7 | Western blot analysis

Cervical DRGs from animals at time points mentioned above were isolated and proteins were extracted using RIPA buffer with protease inhibitors. The samples were solubilized in Laemmli buffer and equal amounts of protein were loaded on 10% precast gel (BioRad Laboratories, Hercules, CA) and transferred to Immobilon membranes. Primary antibodies were incubated overnight [GS (1:10,000), CDH19 (1:200)] and GAPDH (1:25,000) was used as an internal control. HRP-conjugated goat secondary antibodies (1:10,000; Jackson ImmunoResearch) and superSignal West Pico Chemiluminescent substrate (Pierce Biotechnology, Rockford, IL) were used for detection. The signals were normalized using ImageJ software and statistical analysis was done using one-way ANOVA followed by Bonferroni's post hoc test ($n = 2$).

2.8 | Transcriptome analysis

SGCs (fraction 3) and adult SCs were isolated as mentioned and were plated on poly-L-Lysine coated 60 mm dishes (Sigma) and maintained in D10M media for 7 days. Passage 1 SGCs and passage 5 SCs were analyzed in duplicates from two separate experiments for the analysis. RNeasy mini kit (Qiagen, Valencia, CA) was used to isolate RNA and standard procedure was followed. The concentration and purity were measured using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Affymetrix Rat Gene ST 2.0 was used for the transcriptome analysis and only main category probe-sets were used (29,489; Affymetrix, Santa Clara, CA). Transcripts that are $\log_2$ fold change ≥ 2, p value of $\leq .05$ and FDR $\geq .05$ were identified as differentially expressed transcripts.

3 | RESULTS

3.1 | SGCs develop postnatally

Previous studies have utilized both embryonic and adult DRGs as potential sources of SGCs. Figure 1 shows dissociated embryonic (E15) and adult (P90) DRGs at 1DIV. The DRGs are stained with antibodies to β-3 tubulin (TUBB3) to recognize neurons and glutamine synthase (GS) for SGCs. There were several non-neuronal cells in the E15 culture (indicated by arrows) but they neither enveloped the neuronal soma nor expressed GS, the unique marker for SGCs (Figure 1a). In addition to clear increases in neuronal volume (Lawson, Caddy, & Biscoe, 1974), SGCs ensheathing the cell bodies of DRG neurons (indicated by asterisks) were observed only in the adult cultures (Figure 1b), suggesting that SGCs might arise later in development of the PNS. To investigate this idea more closely we examined cervical DRGs from a series of developmental time points for the presence of GS positive cells

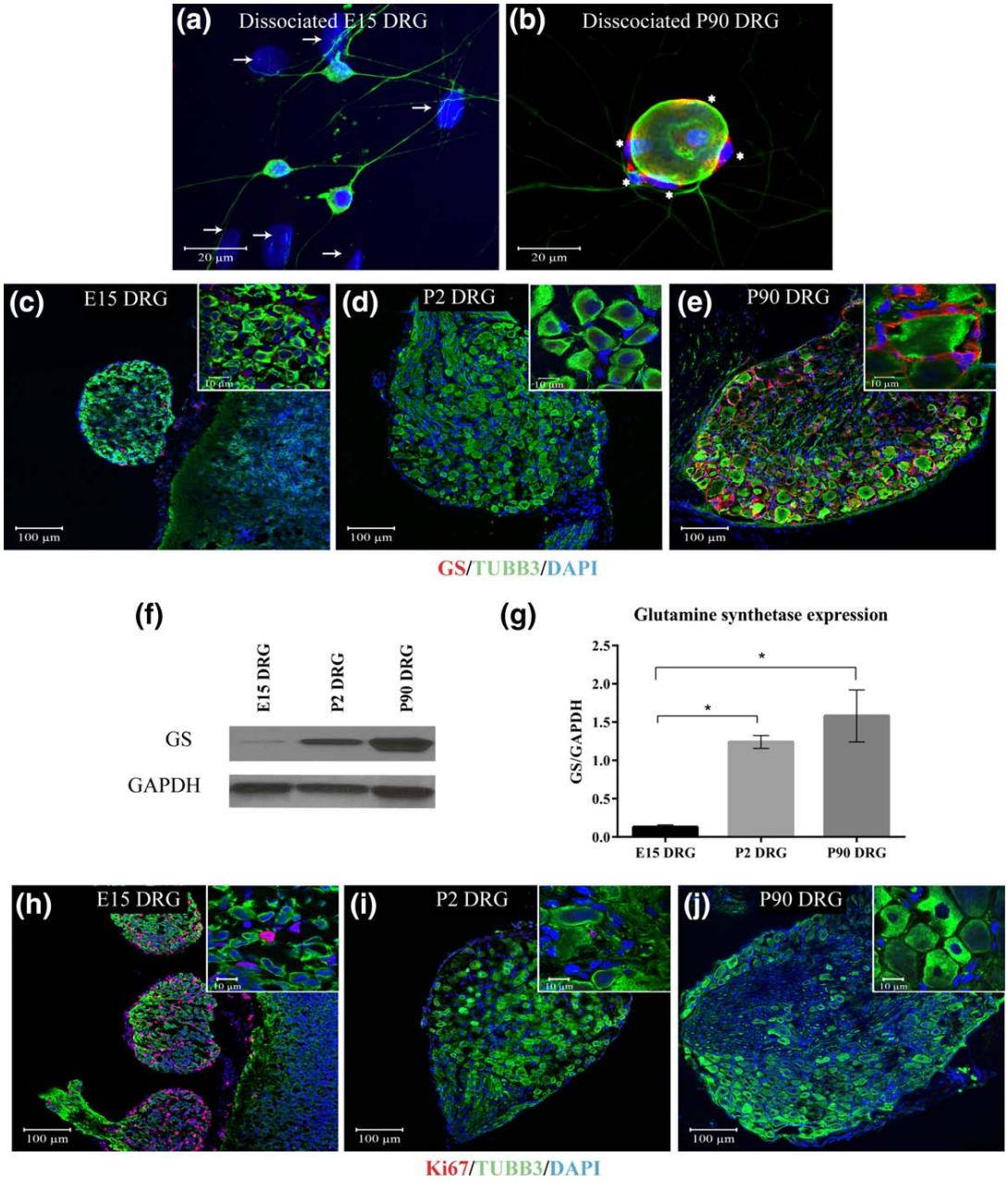

FIGURE 1 Postnatal development of SGCs (a) Dissociated embryonic (E15) and (b) dissociated adult (P90) DRGs at one day in culture showed the presence of several SGCs around the adult neuron (indicated by asterisks) while no SGCs are seen enveloping the embryonic neuron. Arrows point to non-neuronal cells. (c–e) 10 μm sections of E15, P2, and P90 cervical DRGs. (a–e) Cultures were immunostained for GS, TUBB3, and DAPI to identify SGCs, neurons and nuclei respectively. (f) Western blot showing GS expression at the different developmental stages and (g) the quantification of GS expression normalized with GAPDH expression ($n = 2$) showed a significant increase in GS expression in the postnatal developmental stages. Data are presented as mean ± *SEM*. $^*p < .05$. (h–j) 10 μm sections of cervical DRGs identifying proliferative cells by Ki67 staining in E15, P2, and P90 female rats [Color figure can be viewed at wileyonlinelibrary.com]

ensheathing neuronal cell bodies. As can be seen (Figure 1c–e), GS expressing cells appear predominantly in the postnatal period and can be seen surrounding neurons. Analysis by Western blot showed a significant increase in the expression of GS in the postnatal DRGs (Figure 1f–g). This window follows the disappearance of proliferating cells from the developing ganglia as determined by Ki67 staining of the nucleus. Most of the non-neuronal cells of E15 DRG were Ki67$^+$ (Figure 1h) as shown previously by Lawson et al. (1974) and these proliferative cells are absent in P2 and P90 DRGs (Figure 1i–j).

We also observed that we could recapitulate the time course of SGC development *in vitro*. Dissociated E15 DRG cultures were monitored over time for the appearance of GS$^+$ve SGCs, ensheathing the neuronal cell bodies (Figure 2a–c). When we maintained dissociated E15 DRGs for about 5 days, non-neuronal cells started to associate

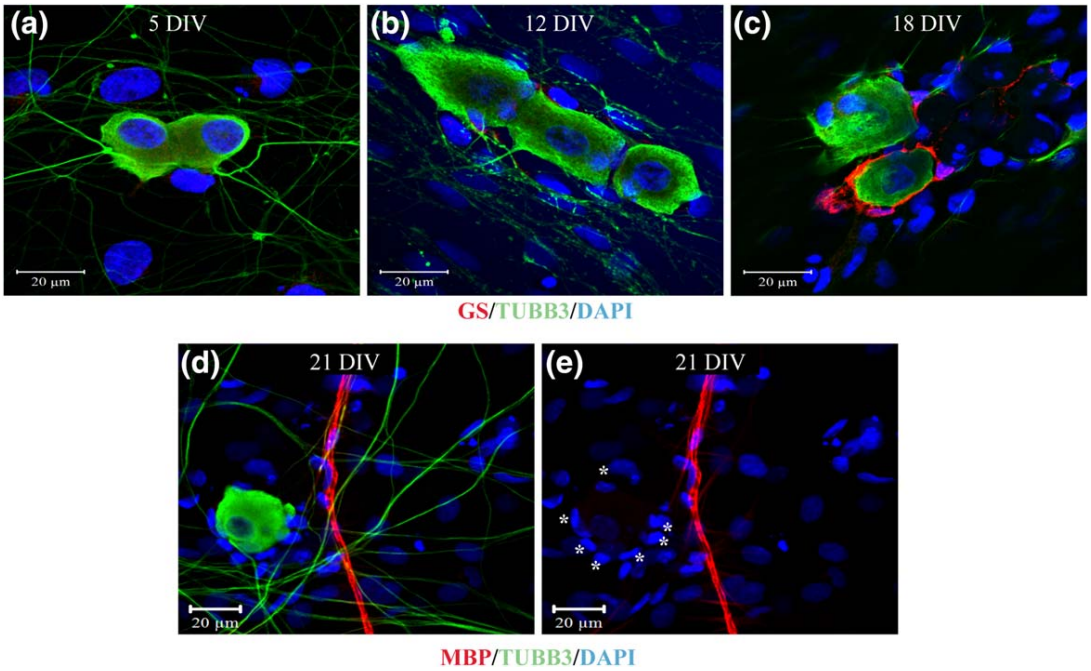

FIGURE 2 Non-neuronal cells within the embryonic DRG are glial precursors. Dissociated embryonic (E15) at (a) 5 DIV (b) 12 DIV and (c) 18 DIV showed an increase in the number of glial cells around the neuron and expression of GS only at later time point. (d and e) Dissociated embryonic culture grown in myelinating conditions (21 DIV) show formation of myelinating SC which is identified by MBP and the cells associating with the neuronal cell body which is indicated by the asterisks. Scale bar represents 20 μm [Color figure can be viewed at wileyonlinelibrary.com]

with the neuronal soma (Figure 2a). By 12 DIV, the number of cells enveloping the neurons increased (Figure 2b) and GS^{+}ve cells were clearly visible by 18 DIV (Figure 2c), correlating with our observations *in vivo*. Interestingly, dissociated E15 DRG cultures have been commonly utilized as a model for PNS myelination (Callizot et al., 2011) where SCs were observed throughout the culture that could be stimulated to form myelin sheaths (identified by myelin basic protein (MBP) upon the addition of ascorbate (Figure 2d,e). Here, we also see SGCs around the neuronal soma (indicated by asterisks). The generation of both SGCs and SCs from E15 DRG support the idea of a common lineage for SC and SGCs in the developing ganglia.

3.2 | SGCs exhibit characteristics of early SCs

To further examine lineage commonalities between SCs and SGCs, we stained cryosections of cervical DRGs with antibodies to markers associated with the SC lineage (Jessen & Mirsky, 2005; Liu et al., 2015). As shown in Figure 3a–e, we observed that adult SGCs expressed CDH19, previously described as a unique marker for SC precursors (Takahashi & Osumi, 2005). Expression of this marker paralleled the development of SGCs observed in Figure 1. We also utilized dissociated adult DRGs at 1 DIV and noted the expression of GAP43 and BFABP (Figure 3f), which have also been associated with the SC precursor stage of development (Britsch et al., 2001; Curtis et al., 1992).

We set out to purify SGCs from adult rat cervical DRGs and developed a method utilizing BSA cushions. Briefly, we harvested the adult DRGs, trimmed the nerve roots and dissociated the ganglia in collagenase. Figure 4a shows the dissociated DRG culture with the several GS^{-} ve cells (indicated by arrows). Once a uniform suspension was obtained, the solution was separated twice on a BSA cushion. The myelin debris and SCs were effectively removed at the end of this separation, leaving neurons with closely adhered SGCs (fraction 1; Figure 4b). This cell suspension was further treated with trypsin solution to further dissociate SGCs from the neurons. BSA cushion separation was performed again to obtain an enriched population of SGCs (fraction 3; Figure 4d) leaving the pellet containing neurons (fraction 2; Figure 4c). SGC population was identified by the expression of GS (we have observed that although the smaller neurons have SGCs ensheathing the cell body, these SGCs do not express GS [Supporting Information Figure S1]) and the neurons were identified by TUBB3 staining. The other non-neuronal cells were identified by DAPI and the absence of both TUBB3 and GS. SGCs were released from the neurons following trypsin treatment (Figure 4f) and as seen in Figure 4d,e, this protocol generated SGCs of greater than 90% purity. When initially cultured, these cells exhibited a flattened fibroblastic morphology (Figure 4g,h). However, after a single passage, the cells appear as bipolar spindles, similar to the shape of a SC (Figure 4i). We see significant differences in cell surface area between passage 0 and passage 1 at 7 days (Figure 4j). Glial marker S100 was used to identify these cells and it is important to note that this morphological change occurred in the absence of axons. Previous studies have also reported that SGCs appear to take on a SC morphology in prolonged culture (Belzer et al., 2010; Poulsen et al., 2014) and we have also observed that cells migrating away from adult DRG explants assume a bipolar spindle shape even after being

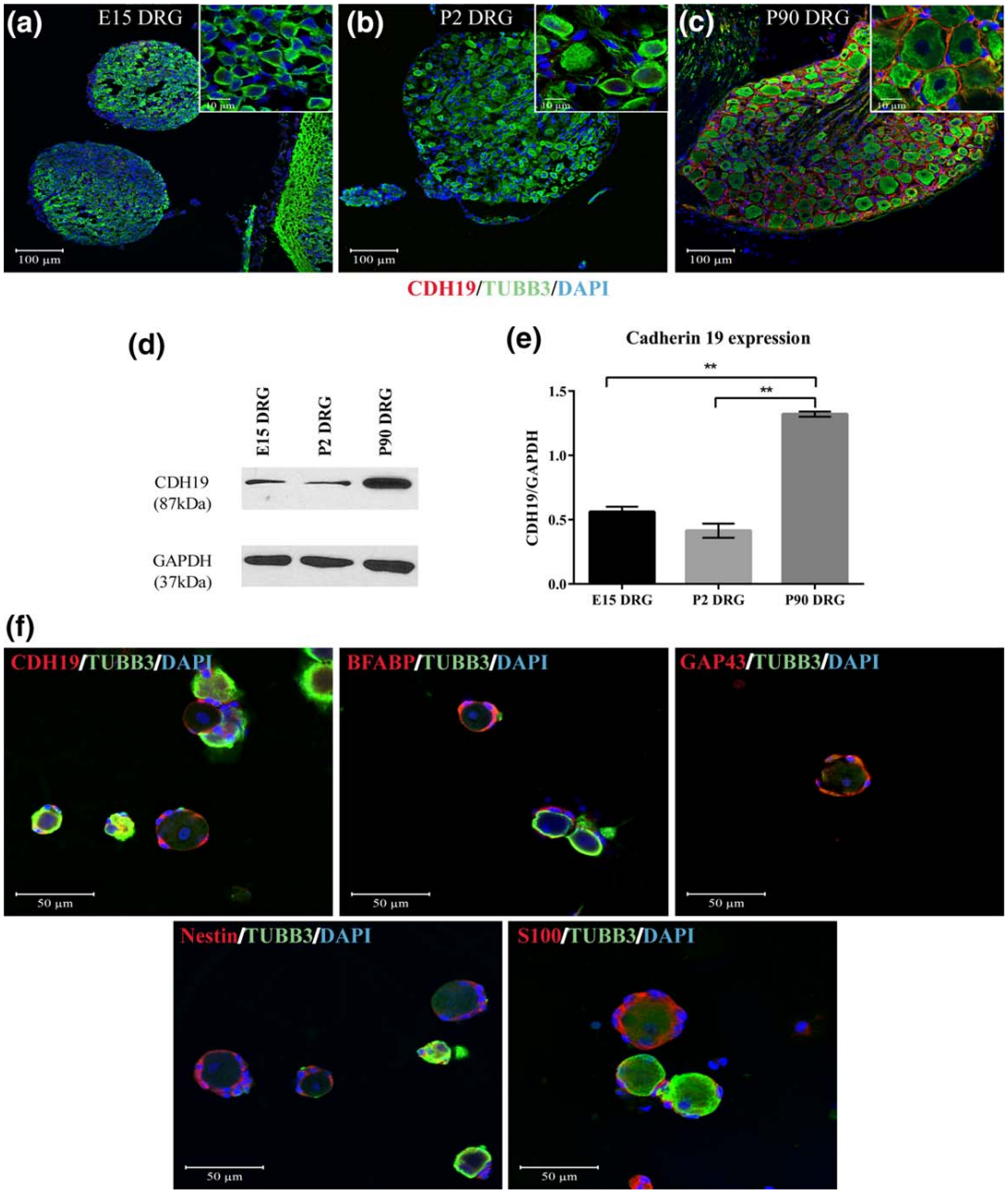

FIGURE 3 SGCs express CDH19. (a–c) 10 μm sections of E15, P2, and P90 cervical DRGs stained for CDH19, TUBB3, and DAPI. (d) Western blot showing CDH19 expression at the different developmental stages (e) the quantification of CDH19 expression normalized with GAPDH expression ($n = 2$) showed a significant increase in CDH19 expression in mature SGCs. Data are presented as mean ± *SEM*. $^{**}p < .01$. (f) Dissociated P90 SGCs at 24 hr after plating stained for CDH19, BFABP, GAP43, Nestin, and S100 [Color figure can be viewed at wileyonlinelibrary.com]

trimmed extensively of nerve roots (Supporting Information Figure S2). We isolated mRNA and performed an analysis of the transcriptome of passage 1 SGCs (Figure 5a) grown in culture for 7 DIV and compared it with the transcriptome of purified passage 5 adult SCs (Figure 5b). As shown in Figure 5c,d, there was a high degree of correlation between the two transcriptomes with less than a 0.2% difference in transcripts analyzed. The expression levels of only 59 transcripts out of the 28,407 analyzed were observed to be significantly different (Figure 5d). The corresponding gene description and the fold change in expression levels between the two cell types are shown in Supporting Information Table S1.

3.3 | SGCs and SCs share similar functional properties

Given the striking similarities between SGCs and SCs we evaluated whether purified SGCs were functionally similar to SCs, by evaluating

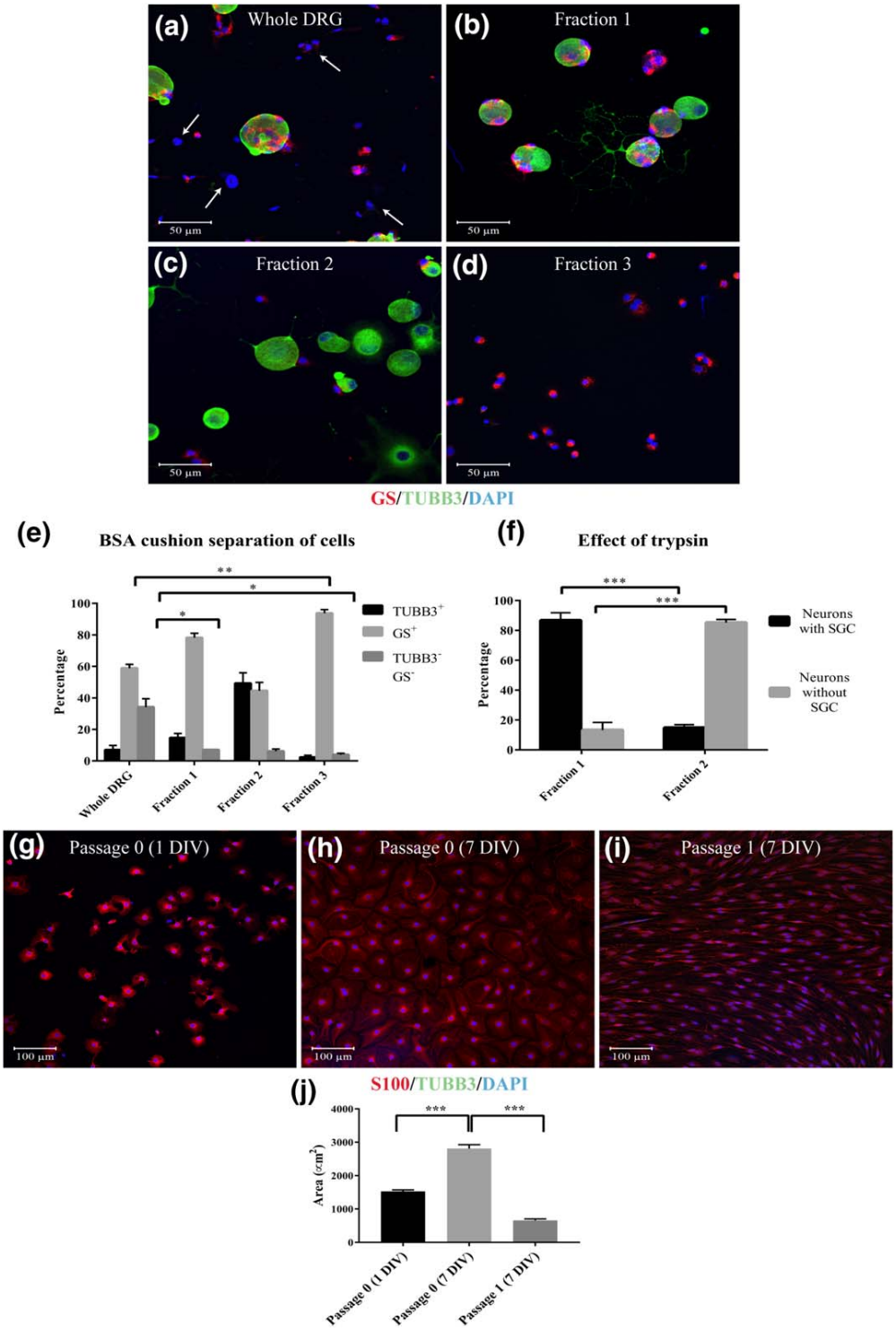

FIGURE 4 Isolation of SGCs and morphological changes of SGCs. (a) P90 DRGs treated with collagenase and plated prior to BSA separation. Arrows identify glial cells that are GS negative. (b) The pellet after separation on BSA cushion. (c) Pellet and (d) supernatant after further treatment with trypsin followed by separation on BSA cushion. SGCs are identified by GS, neurons by TUBB3 and other glial cells are identified by the absence of both markers. (e) Percentage of different cell types in whole dissociated ganglia and in each of the fractions ($n = 3$). (f) Effect of trypsin on SGC separation from the neurons. Data are presented as mean ± *SEM*. $^{*}p < .05$. Isolated SGCs at (g) 1 day after plating (h) 7 days after plating and (i) 7 DIV after one passage (P1). (j) Quantification of the cell surface area between P0 (1DIV, 7DIV) and P1 (7DIV) 100 cells were measured from each condition. Data are presented as mean ± *SEM*. $^{***}p < .001$ Cells are identified by S100 staining and nuclei by DAPI. Images indicate a morphological change from a flattened fan shape to a bipolar spindle shape after passaging [Color figure can be viewed at wileyonlinelibrary.com]

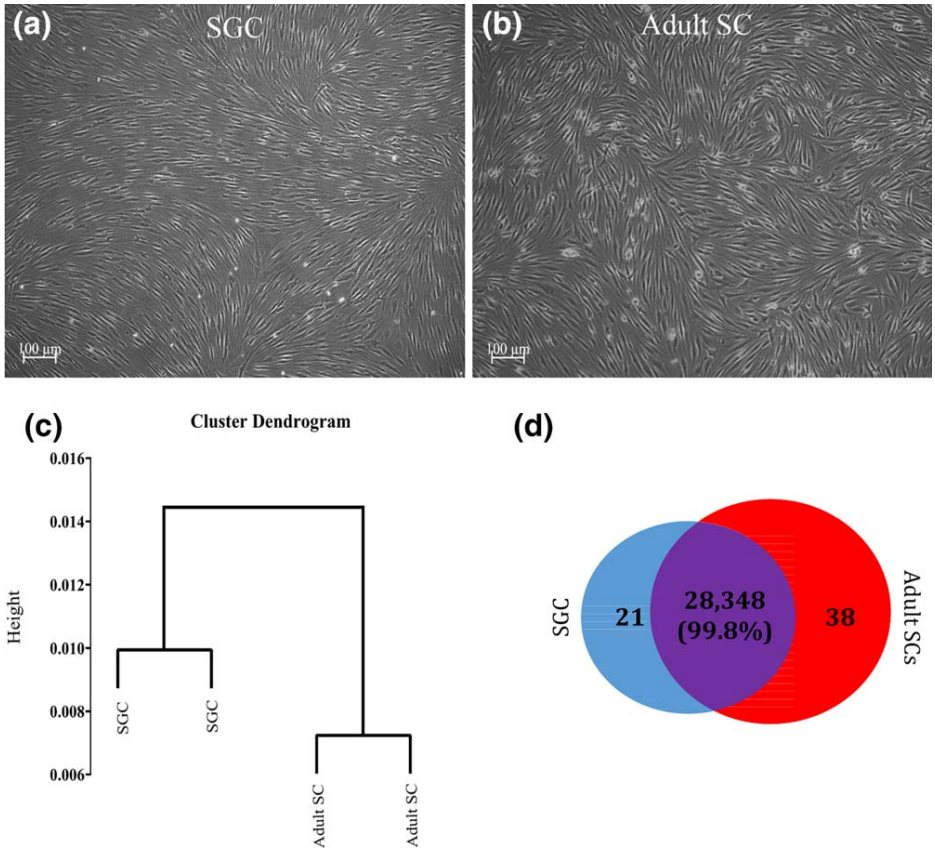

FIGURE 5 SGCs and SCs are closely related cell types. (a) Isolated passage 1 SGCs and (b) passage 5 adult SCs grown in similar conditions at 7 DIV. (c) Dendrogram of Affymetrix Rat Gene 2.0 array comparison between the replicates of each cell type and between the two different cell types show a high degree of correlation. (d) Illustration showing the number of transcripts that are differentially expressed between the two cell types [Color figure can be viewed at wileyonlinelibrary.com]

whether they were capable of myelinating sensory axons. Initially we utilized adult cervical DRGs, as an intact explant (Figure 6a). Switching this culture to a myelin permissive feed for 21 days (35 DIV) allowed the formation of multiple myelin segments as evidenced by staining for MBP. To our knowledge this is the first time that adult axons have been myelinated in culture. As it is possible that the explant contains a number of SCs that could be responsible for the observed myelin segments, we carried out the same experiments using neurons, with their ensheathing SGCs intact, that have been purified on a BSA cushion (fraction 1, Figure 4b). As is observed in Figure 4b,e, this process significantly reduces the numbers of non SGC cells (defined as [TUBB3$^-$, GS$^-$]) present in the preparation. Again, multiple myelin segments were observed when the preparation was switched to myelin feed (Figure 6b). Finally, we used a classic myelinating coculture, utilizing E15 DRG neurons that have been purified to remove non-neuronal cells. After developing extensive axonal processes, BSA cushion purified SGCs (fraction 3, Figure 4d) and adult rat SCs purified from the sciatic nerve, were added to the neurons and cocultured as mentioned before being switched to a myelin permissive media. As is seen in Figure 6c,d, both SGCs and adult SCs were capable of forming myelin segments on these axons. Interestingly, although SGCs produced collagen, they failed to assemble it into arrays along the axon (Supporting Information Figure S3).

4 | DISCUSSION

In this study, we have examined the development of SGCs, cells that ensheathe sensory neuron soma. We have shown that these cells arise postnatally and express early SC markers such as CDH19, which has previously been described as a unique marker for SC precursors (Takahashi & Osumi, 2005). To examine the relationship between SGCs and SCs, we developed a novel method for the purification of SGCs and showed that these cells are transcriptionally and morphologically similar to SCs even in the absence of axonal contacts. Finally, we were able to assess how functionally similar SGCs are to SCs by demonstrating that purified SGCs were able to myelinate sensory axons in both organotypic and mixed cocultures. Based on these observations we hypothesize that SGCs represent a population of SC precursors whose further development has been arrested as a result of the ganglia microenvironment and/or contact with neuronal soma.

CDH19 has been described as the only unique marker for SC precursors (Takahashi & Osumi, 2005) and this marker is down regulated as these precursors differentiate into immature SCs in response to axonal contact. In this study we show that "adult" SGCs that surround neuronal cell bodies also express CDH19 and that, when purified, these cells adopt the characteristic bipolar spindle cell shape of the SC, even

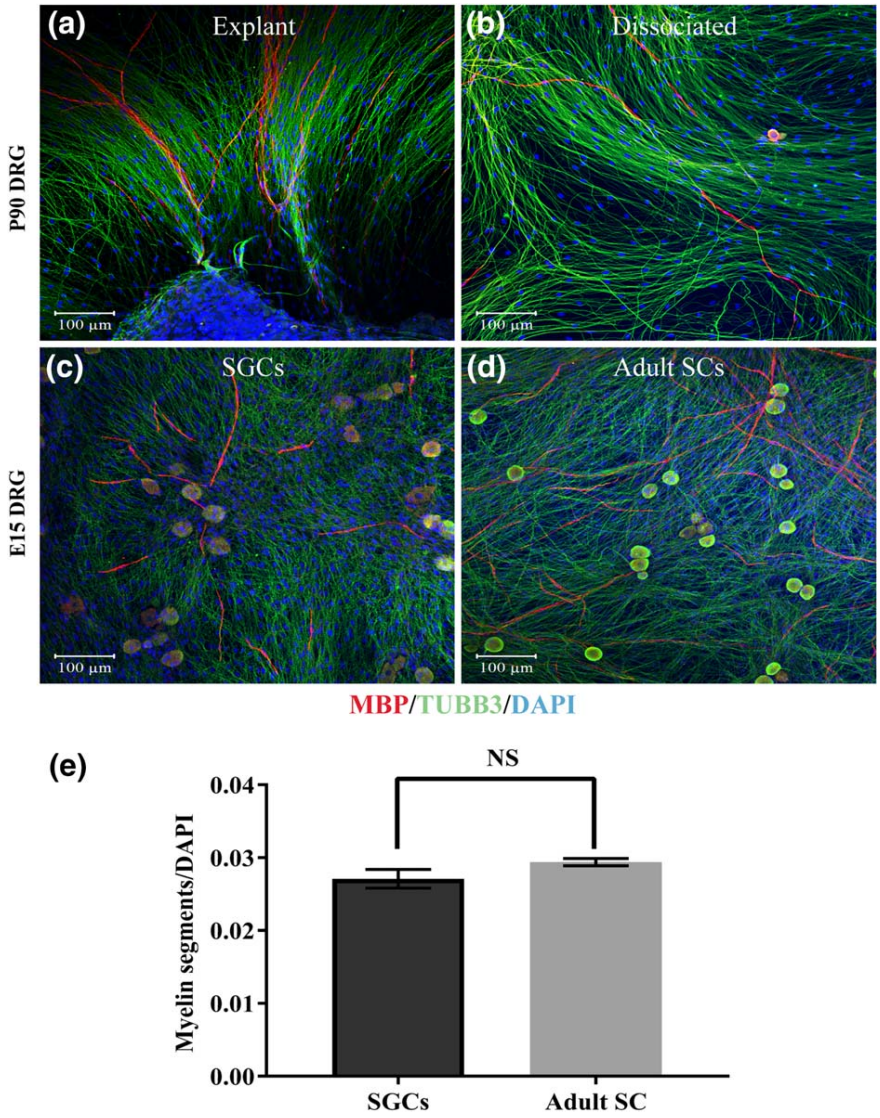

FIGURE 6 SGCs can myelinate both embryonic and adult axons. (a) P90 DRG maintained as an explant and (b) dissociated P90 DRG (fraction 2) at 35 DIV in myelin permissive conditions. Purified E15 neurons cocultured with (c) SGCs and (d) adult SCs at 40 DIV. Myelin segments are identified by MBP and neurons by TUBB3. Scale bar represents 100 µm. (e) The number of segments formed by SGCs and SCs normalized with DAPI count. Data were analyzed by unpaired *t* test ($n = 3$) and presented as mean ± *SEM* [Color figure can be viewed at wileyonlinelibrary.com]

in the absence of axonal contact, to become functional myelinating cells. Recent studies indicate that in addition to SCs, SC precursors can also give rise to parasympathetic neurons (Dyachuk et al., 2014; Espinosa-Medina et al., 2014), suggesting that these cells exhibit a degree of plasticity outside of the SC lineage. SGCs have also been described as being potentially plastic and have been proposed to give rise to a number of different cell types including sensory neurons and even oligodendrocytes (Fex Svenningsen et al., 2004). It has been proposed that SGCs may represent a reservoir of multipotent cells that could replenish damaged neurons within the ganglia. Our study supports this idea by demonstrating that SGCs exhibit properties of SC precursors.

The differentiation of SC precursors to immature SCs and finally myelinating SCs has been well characterized in the context of the developing peripheral nerve (Jessen & Mirsky, 2005; Mirsky et al., 2008). In those studies the SC precursor has been shown to require the neuregulin growth factor for its survival, propagation and differentiation into an immature SC. *In vivo*, this is provided by contact of the SC precursor with the axonal bound neuregulin. In our studies we note that markers such as CDH19 are quickly downregulated once SGCs are no longer in contact with neuronal soma and that SGCs quickly adopt a SC morphology when placed in culture. There these cells can be propagated in the absence of neuregulin or axonal contact and can be added to DRG neurons to form myelin segments. It should also be noted that

they exhibit a different pattern of ECM deposition, and appear less organized than their counterparts derived from purified adult rat SCs. We believe that this may reflect differences in the differentiation of SGCs to a myelinating cell type compared with SC precursors or simply technical differences resulting from the preparation of SGCs.

It is well-established that the embryonic DRG is a source for the generation of viable population of SCs (Wood, 1976), and several previous studies (Fex Svenningsen et al., 2004; Maro et al., 2004; Wakamatsu, Maynard, & Weston, 2000; Weider et al., 2015) have referred to non-neuronal cells within the embryonic DRG as SGCs. Although these cells lie within the DRG (where neuronal cell bodies are clustered), we have shown that these cells in the embryonic DRG are not yet mature SGCs as they neither envelope the neuronal cell body nor express characteristic SGC markers such as GS. This observation suggests that these non-neuronal embryonic cells might represent an earlier neural crest derivative. In the first two weeks of the postnatal period, Ki67 staining suggests that most cells in the ganglia have ceased proliferation and are starting to differentiate into SGCs in close proximity with the neuronal soma.

The role of CDH19 in SGC function remains to be determined. When adult DRGs, complete with their ensheathment of SGCs are placed into culture, CDH19 is quickly downregulated, concomitant with the migration of SGCs away from the neuronal cell body (unpublished observations), suggesting a potential role for CDH19 in maintaining SGC–somal/SGC–SGC interactions. In support of this idea, it is interesting to note that the distance between neuronal surface and SGCs is 20 nm (Pannese, 1981) which is comparable with the distance between cells in the cadherin-based adherens junction. However, we have also noted that adult SCs, which do not appear to express CDH19, are capable of ensheathing mature DRG soma (unpublished observations) suggesting that, other unidentified adhesion molecules may also play a role in the SGC/neuronal cell body interaction. Although these SCs can ensheathe the neuronal cell body they do not express SGC markers such as GS.

In this study, we purified SGCs from adult rat DRGs and demonstrated their ability to myelinate purified sensory neurons. We were aware of the presence of contaminating cells (including SCs) within the harvested adult ganglion. However, in the method used to isolate SGCs, we typically obtained about 90% SGCs and a small percentage of neurons. The neurons failed to attach and thrive on PLL coated surfaces and passaging resulted in their complete loss. We also observed SCs (GS^{-}ve, $TUBB3^{-}$ve) but noted a fivefold reduction in SC numbers when the separation was performed, with the total percentage of SCs in fraction 3 less than five percent. It is unlikely that this small percentage of SCs in culture contribute to the formation of myelin segments observed in the cocultures experiments.

SGCs have been proposed to regulate the electrical activity of DRGs and have been shown to undergo several changes during nerve injury that are thought to underlie the recruitment of additional neurons to an injury response (Hanani et al., 2002; Ohara, Vit, Bhargava, & Jasmin, 2008; Pannese, Ledda, Cherkas, Huang, & Hanani, 2003; Siemionow, Klimczak, Brzezicki, Siemionow, & McLain, 2009; Stephenson & Byers, 1995; Vit, Jasmin, Bhargava, & Ohara, 2006). Recent progress has been made in understanding the signaling exchange between the SGC and the neuronal soma as well as between adjacent SGCs, with the idea that perturbing these pathways can lead to the development of novel therapeutics for dealing with neuropathic pain. Greater understanding of the differentiation of the SGC as proposed in this study could facilitate the development of such therapeutics.

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SUPPORTING INFORMATION

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