Brief summary

Schira-Heinen and colleagues focused on the process of transdifferentiation of Schwann cells that gives rise to the so-called Repair Schwann cells, which are essential to activate a repair programme for the functional regeneration of peripheral nerves after injury. The mechanisms involved in this process are not yet fully understood, and the authors therefore decided to investigate the role of the sphingosine-1-phosphate receptor (S1PR). Indeed, Fingolimod, an S1P receptor agonist, has recently been shown to enhance the repair phenotype of Schwann cells through the activation of dedifferentiation markers and the concomitant release of trophic factors resulting in increased neurite outgrowth. But, the main SP1 receptor involved in the process is not known. In vitro results obtained in this work, using primary neonatal cultures of rat Schwann cells and a plethora of agonists and antagonists specific for one of the five S1PRs (S1P1, S1P3-5) revealed that S1P3 and S1P1 represent the main receptors responsible for Schwann cell transdifferentiation. The authors conclude that in vivo modulation of S1P1 and S1P3 could be effective in enhancing the repair phenotype of Schwann cells and thus stimulate proper nerve repair.

General Comments

The manuscript, although clearly written and quite relevant to the field, is not adequately presented and needs further experimental confirmation. The work was mainly conducted in vitro and using only the technique of real time PCR to assess the expression of receptors and certain intracellular targets activated following stimulation of these with agonists and antagonists.

Major Revisions

1) I believe that talking about expression of certain receptors using only the real time PCR technique is not completely adequate as post-translational modifications could affect receptor expression. Therefore, assays such as Western blot and immunofluorescence analysis to verify the actual expression and membrane localisation of receptors are necessary for the message of the work. Since specific antibodies for the different receptors are commercially available I ask that this will be verified, at least for SP1 and SP3 receptors.

2) In Figure 1, only the SP3 receptor is shown to be statistically significant. SP2 is not significant? In general, in every graph where the statistic is not present is it because the data is not significant? This, should be added and specified in the figure legends.

3) In figures 2G and 4G housekeeping (such as GAPDH or actin) control is missing for quantification of cJun and PTEN. Even if authors in MM say they loaded the same amount of protein in my opinion a housekeeping protein is necessary

4) In line 328-329 the authors argue that Fingolimod mediated induction of p-cJUN is likely to be S1P3. This aspect is not completely clear to me. First, as mentioned above, western blot analyses need to be normalised with housekeeping proteins. In addition, Fingolimod appears to promote both SP1R- and SP3R-dependent cJUN synthesis (Fig 2H and 4H). Furthermore, p-cJUN appears to be dependent on both receptors as the use of specific antagonists causes reduced expression of p-cJUN after treatment with Fingolimod (Fig2K and Fig4K) even if statistical analysis is missing (or is not significant).
Therefore, these results need a better explanation.

5) Previous results have revealed that S1P induces remodelling of the actin cytoskeleton in Schwann cells, promoting cell migration. These are essential processes for Repairs Schwann cells.

It would be interesting to test whether Fingolimod induces a similar morphological change (with IF analysis)

6) In Kohne et al., 2012, the authors of Arch Neuro showed that lower concentrations of Fingolimod (10nM and 100nM) showed no effect on cell proliferation or viability, but increased activation of caspase 3 and caspase 7 (two markers of apoptosis). In contrast, 1 μM FTY720P showed a decrease in cell proliferation and viability. The different agonists used in the work were employed at a micromolar concentration. Did authors test whether these concentrations impaired cell viability?

Minor Revisions

Lines 34-37 need citations. I suggest Jessen KR, Arthur-Farraj P (2019), Glia, 67, 421-437 and Rigoni M, Negro S (2020) Signals Orchestrating Peripheral Nerve Repair. Cells, 9.

Although the aim of the work is to understand which receptor is responsible for Schwann cell transdifferentiation after Fingolimod treatment, I would discuss, in more detail, (in the discussion section) the role of the natural ligand SP1 in the process. Is SP1 express in vivo after nerve injury? If yes, which cells express it? Is it the axis SP1-SP receptor involved in Peripheral nerve regeneration?

Lines 66-67 the sentence “Additionally, the TGFβ-Smad signaling pathway is activated in response to Fingolimod treatment” needs reference

Line 75, sentence “Since Fingolimod targets four out of five S1PRs (S1P1, S1P3-5)” needs references.