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YURI DA ROSA RIGO

O POTENCIAL NEUROGÊNICO DA NG2-GLIA NO NEUROTRAUMA: UMA REVISÃO SISTEMÁTICA.

Porto Alegre

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Trabalho de Conclusão de curso apresentado como requisito parcial para obtenção do título de Bacharel em Biotecnologia com ênfase em biotecnologia molecular na Universidade Federal do Rio Grande do Sul. Manuscrito formatado segundo as regras editorias da Neuroscience & Biobehavioral Reviews.

Orientador(a): Prof. Dr. Luis Valmor Cruz Portela

Co-orientador(a): Prof. Dr. Nathan Ryzewski Strogulski

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Prof. Dr. Luis Valmor Cruz Portela - Orientador

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Prof. Dr. Nathan Ryzewski Strogulski - Co-orientador

Prof. Dr. Jean Pierre Oses - FURG

Prof.ª Dr.ª Ana Paula Herman - UFRGS

Resumo

Neurotrauma é uma patologia preocupante para a sociedade devido à sua alta ocorrência somada com a dificuldade de precaução e tratamento das sequelas motoras e cognitivas resultantes. Morte neuronal e resposta glial imediata reprimem o desenvolvimento de tratamentos regenerativos ao tecido nervoso focando sintomas de curto prazo, desviando os esforços médicos para a contenção do alastramento do dano tecidual e moderação dos sintomas de longo prazo. Pesquisas constataram que a célula da glia “NG2-glia” possui, dentre outras características exclusivas, a capacidade inata de diferenciação em oligodendrócito e o potencial de diferenciação em neurônios, instigando pesquisas que busquem possíveis manipulações que promovam tal potencial. Aqui, foi realizada uma revisão sistemática resumindo os artigos presentes que exploram o potencial neurogênico das células NG2 em modelos de neurotrauma e neles foram constatadas diversas manipulações que obtiveram sucesso nesse fomento neurogênico tanto em casos de trauma cranioencefálico como de trauma raquimedular. Assim, foi possível projetar estratégias para futuros estudos no tópico que visem aplicações clínicas e a supressão dos danos causados pelo trauma.

Introdução geral

Lesões mecânicas no Sistema Nervoso Central (SNC) são majoritariamente classificados em dois grupos: Trauma Cranioencefálico (TCE) e Trauma Raquimedular (TRM). TCE é definido por qualquer lesão física que impeça o funcionamento correto do cérebro e é listado - segundo o centro de prevenção e controle de doenças norte-americano (www.cdc.gov) - como a principal causa de morte em pessoas com idades entre 5 e 44 anos, sendo responsável por 10% do total de mortes no mundo. Quanto às vítimas que sobrevivem, calcula-se que 43% delas mantém pelo menos uma sequela, das quais as mais comumente observadas são depressão, ansiedade, agressão e paranoia (Haarbauer-Krupa

et al., 2021). Segundo o DATASUS TabWin (www.datasus.gov.br), houveram 547.468 casos de TCE no Brasil em 2011 com uma tendência crescente quando projetado para anos futuros. TRM é definido por qualquer disfunção da medula espinhal devido à danos físicos e, igualmente ao TCE, tem como principais causas ocorrências comuns como quedas, acidentes de trânsito e agressões (Capizzi et al., 2020; Shao et al., 2019) além múltiplas correlações de concussão derivada de esportes com sintomas de longo prazo de neurotrauma (Manley et al., 2017).

Do ponto de vista molecular, em um cenário de neurotrauma há a liberação do citosol das células neuronais e gliais rompidas durante a lesão. Essa sobrecarga de sinais químicos é altamente tóxica para o tecido e é interpretado pela glia como indicativo de neuroinflamação, desencadeando assim uma gama de respostas que, em conjunto, dão início à gliose (Pekny and Nilsson, 2005). A gliose é uma resposta defensiva aguda com o objetivo de suprimir o alastramento de cascatas neurodegenerativas e para tal utiliza, como uma das principais estratégias, a síntese da cicatriz glial (Sofroniew, 2009). Apesar da importância biológica desses processos citados, a cicatrização rápida e inata - somada com a incapacidade de neurogênese do órgão adulto respectivo - dificulta o desenvolvimento de tratamentos com o intuito de promover a regeneração do tecido perdido perante o trauma (Sofroniew, 2009).

Uma das classes de células que compõem a glia é a NG2-Glia (também descrita como “Célula NG2” ou “Célula Precursora de Oligodendrócitos” ou apenas por “CPO”). Ela é a quinta classe mais comum do tecido e a mais proliferativa, compondo 3% da matéria branca e 9% da cinzenta (Eugenín-von Bernhardi and Dimou, 2016). Estudos das últimas duas décadas concluíram colaborativamente que essa classe possui características exclusivas dentre as do tecido nervoso como: homogeneidade; capacidade proliferativa que mantém essa homogeneidade e aumenta sua densidade quando reativa; capacidade inata de reação a lesões; e capacidade inata de diferenciação em oligodendrócitos quando reativa (Eugenín-von Bernhardi and Dimou, 2016; Robins and Kokoeva, 2018). Tais características fomentam pesquisas quanto a um possível potencial neurogênico. Entretanto, não houve concordância de tal potencial entre eles e os estudos mais otimistas reportaram apenas neuro-diferenciações diminutas

das células NG2 (Dimou et al., 2008; Kang et al., 2010; Rivers et al., 2008). Esses resultados demonstram uma incapacidade inata de neuro-diferenciação, mas não excluem a possibilidade de manipulações que a promovam.

Devido ao impacto social da neurodegeneração causada por neurotrauma e sua dificuldade de tratamento; e às características exclusivas das células NG2 e seu potencial neurogênico; o objetivo desse trabalho é revisar sistematicamente estudos que exploram manipulações neurogênicas em células NG2 aplicadas a modelos de neurotrauma.

The neurogenic potential of NG2-Glia in Neurotrauma: a systematic review

Yuri da Rosa Rigo[A], Radharani Benvenutti[B], Luis Valmor Portela[A], Nathan R Strogulski[A*]

[A] Neurotrauma and Biomarkers Laboratory, Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre, Brazil
[B] Laboratório de Psicofarmacologia e Comportamento, PPG Neurociências, ICBS, UFRGS, Porto Alegre, Brazil

[*]Correspondence to:
Nathan R Strogulski, Neurotrauma and Biomarkers Laboratory, Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre, Brazil. Rua Ramiro Barcelos 2600, Anexo Bioquímica, zip-code 90035-003. E-mail: n.strogulski@gmail.com

ABSTRACT

Regenerative approaches towards neuronal loss after a traumatic brain or spinal cord injury are a technological challenge and a dogma in neuroscience. Current knowledge regarding NG2 cells indicates that they may be capable of differentiating into neurons. As these cells show great potential to take a regenerative role, studies have been made to try different manipulations aiming at a neurogenic response from them in trauma models. Here, we systematically review these articles to summarize their findings and to project future directions with what is known about these cells. It was summarized that NG2 cells have a manipulatable neurogenic potential that can be explored by pharmacological and genetic approaches and that they respond well to ex-vivo transplantations, extending what can be researched for future clinical treatments.

Keywords: *neurogenic, differentiation, NG2, TBI, SCI, trauma*

Highlights

- Neurogenic potential of the NG2 cells.
- Developing treatments for neurotrauma.
- Genetically manipulation of NG2 cells.
- Glial transplantation as treatment.

1. INTRODUCTION

Mechanical injuries to the Central Nervous System (CNS) are major medical conditions and leading causes of mortality and disability in the world, caused by common occurrences like violence, traffic accidents, and falls (Capizzi et al., 2020; Shao et al., 2019). Traumatic injuries to the nervous system, hereafter called neurotrauma, are associated with acute mechanical damage to the brain or medullary tissues - traumatic brain injury (TBI), and spinal cord injury (SCI), respectively - that leads to the rupture of cell membranes and release of cytosolic content, and also axonal stretching and neuronal excitotoxicity (Pekny and Nilsson, 2005; Sofroniew, 2009). Following these acute events of an injury, a myriad of adaptive biochemical cascades and neuroimmune responses ensue to promote brain/spinal cord tissue recovery and repair, which promote an attenuation of acute disabilities following lesions, such as motor and memory dysfunctions (Chang and Badjatia, 2014). Nonetheless, these mechanisms also contribute in the long-term as substrates to neurodegeneration, associated with the increased incidence of dementia and motor disorders in TBI and SCI patients (McKee et al., 2015).

Historically, most research towards therapy in TBI and SCI has focused on secondary damages, in spite of the primary events resulting from injury, particularly due to the apparent irreversibility of acute neuronal loss. Although current knowledge regarding neurogenesis is challenging this concept, some obstacles remain in the frontiers of neuroregeneration and repair (Nagappan et al., 2020). For instance, physiological neurogenesis is restricted to a few regions in the adult CNS (Braun and Jessberger, 2014; Fares et al., 2019; Flor-García et al., 2020); adult human neurons are not able to replicate and are part of a very complex and delicate system (Aranda-Anzaldo and Dent, 2017); to date, no safe clinical treatments to revert neuronal loss have been approved, only to decelerate its propagation (Khellaf et al., 2019). Albeit, considering the immense potential of promoting acutely not only the improvement of brain homeostasis but actual repair and recovery of lost neural connectivity, therapies targeting neurogenesis in TBI and SCI are paramount to improve short-term

recovery and also long-term prognostic of consciousness, memory, and cognitive skills, required for social reinstatement of these patients.

NG2 cells - or Oligodendrocyte Precursor Cells (OPC) - are one group of glial cells that are, unusually, motile and homogenous in the CNS (Eugenín-von Bernhardi and Dimou, 2016). They respond to injury dividing and migrating to the affected site - guided by the excess of glutamate (Robins and Kokoeva, 2018) - to participate in the glial scar formation and to differentiate into oligodendrocytes (Scheller et al., 2017) with some studies even suggesting that a small percentage differentiate into astrocytes (Alonso, 2005; Tatsumi et al., 2008). Their response is vital for the repair of the tissue and NG2 cells have an important role in gliosis and in the maintenance of the nervous (Eugenín-von Bernhardi and Dimou, 2016). Being a precursor cell and, therefore, naturally being able to differentiate under the right circumstances, tests to evaluate the percentage of NG2 cells that can turn into cells displaying markers and/or the physiologic anatomy of neurons were made with huge scopes to use their - so far uncertain - capabilities as possible treatments in TBI/SCI/Degenerative scenarios (Aguirre et al., 2004; Geha et al., 2010; Guo et al., 2009). Unfortunately, there is too much disagreement in whether these cells can or cannot give rise to new neurons as a response to physiological or pathological conditions, due to the divergent results in the literature. Moreover, even in works with favorable outcomes, the percentage of NG2 cells that showed neuronal markers was too low for future clinical treatments (Dimou et al., 2008; Kang et al., 2010; Rivers et al., 2008), limiting the translation capacity of this technology.

However, being gliosis a major acute response to injury at the CNS and being NG2 cells capable of dividing, migrating, to keep their homogeneity and differentiate within gliosis, multiple approaches were made to these cells aiming neurogenic differentiation after some type of manipulation. In this study, we systematically review all research present in the selected databases that investigate neurogenic inductions to NG2 cells applied in Traumatic Brain Injury (TBI) or Spinal Cord Injury (SCI) models to answer if these cells have the potential to become the main role of a future treatment for neurotrauma.

2. REVIEW CRITERIA, SCREENING RESULTS AND RISK OF BIAS

A systematic review according to the SYRCLE (www.syrcle.network) model was conducted utilizing the databases Pubmed/MEDLINE, Web of Science, and SCOPUS. The project of this work was previously published at the platform Open Science Framework (www.osf.io; DOI: 10.17605/OSF.IO/B5SPU). The terms used to perform the search are available at Supplementary Material 1. All searches were conducted on April 23, 2021, by two independent reviewers (Rigo and Strogulski) with a third (Benvenutti) for cases of discrepancies, utilizing only studies that were published between January 1, 1998 (as no studies were found before that date with the selected search terms), to April 23, 2021. There were a total of 2703 studies collected of which 1997 of them were found to be duplicates after detailed inspection using Rayyan (www.rayyan.ai), resulting in 1504 studies selected for screening. Next, an initial screening based on the title and abstract was done before a second full-text screening for the remaining papers. 24 articles were selected for full-text evaluation where the inclusion criteria were: Peer-reviewed research articles describing preclinical experiment studies, studies that investigate NG2 cells associated with neurogenesis; studies that investigate neurotrauma; studies that investigate models *in vitro* and/or *in vivo*; and the exclusion criteria were: non-English studies; review, commentary, conference proceedings, and corrections publications; studies done on humans; *in silica* studies; studies that don't explore neurotrauma models and studies that don't explore the neurogenic potential of NG2 cells.

Finally, 11 articles were selected for this review. The flow chart (Figure 1) summarizes all screening stages that resulted in the included articles. All screening stages were executed by two independent reviewers (Rigo and Benvenutti) with a third (Strogulski) for cases that presented discrepancies.

FIGURE 1

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only

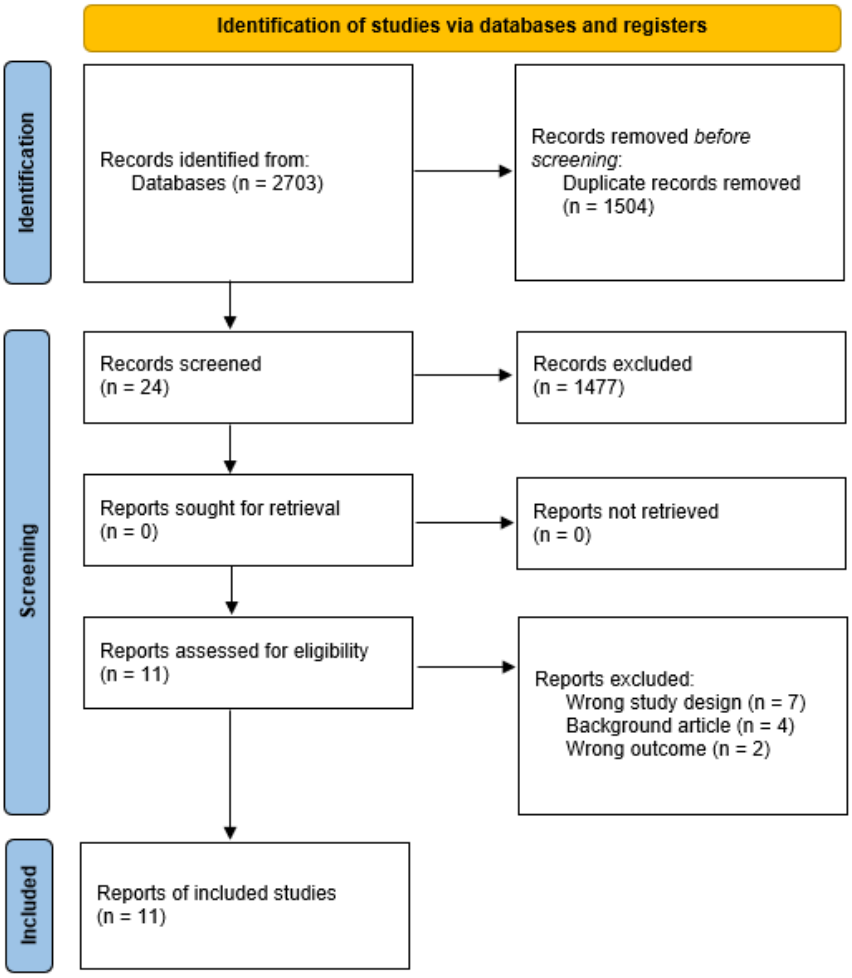

Figure 1: Flow chart of the inclusion of studies for the systematic review.

A table of relevant - for this review - experiments and its details was made with the final selected studies to better compare their methodologies and outcomes (table available at Supplementary Material 2). Utilizing suggested questions (Hooijmans et al., 2014) and the table created, it was made a risk of bias chart (Figure 2).

Figure 2

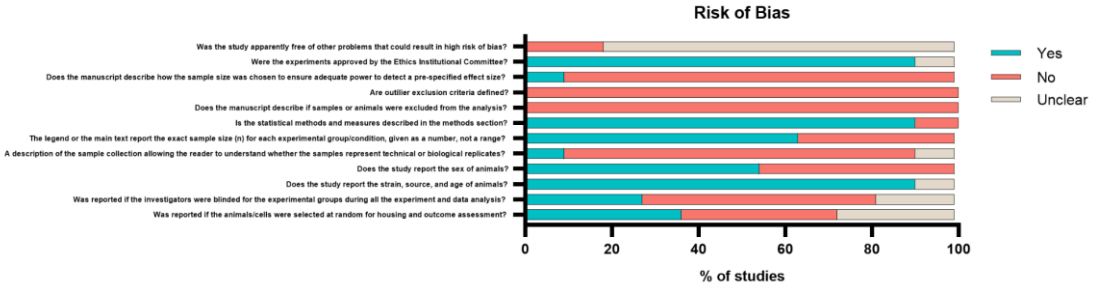

Figure 2. Risk of bias assessment and reporting questions on randomization, blinding, and power calculation of the included studies.

It is worth noting that these papers presented a high risk of bias with a lot of them being absent about important descriptions of their methods, adding concerns about their respective approaches. The majority of the studies doesn't describe how the sample size was chosen, if their tests were made with blind or double-blind manners and sometimes even the sex of the animals used wasn't clear and none of them explain how they manage to exclude or if they even had outliers in their results. This is a reflection of the issue that many fields in the scientific community face with the reproducibility and replicability of complex methodologies, such as animal models for trauma, that have numerous hidden variables. To enhance the reliability of the generated data and the reproducibility of the study it is demanded that researchers detail their methodologies in the published manuscript. As the risk of bias was presented as high, the number of studies being selected was low and minimum criteria was missing, it was chosen to not do a metanalysis with the subject.

3. GENERAL RESULTS

In our systematic review, it was found a homogenous division of studies investigating TBI (4 studies) and SCI (7 studies) as indicated in Figure 3A, and therefore organized our findings within these two major subgroups. However, there is a substantial degree of heterogeneity in the methodological approaches employed to promote neuronal recovery following injury, ranging from endogenous signalling of the injured CNS, pharmacological inducers to genetic

modification of NG2 cells, as indicated in Figure 3B. Although this may be a reflection of the pioneering nature of these works, it does allow a broader understanding of both the potential culprits for acute neuroregenerative therapy using genetic and pharmacological approaches and also the pathobiological conditions that follow an injury. With that in mind, sessions were further stratified by the kind of neurogenic induction within these major subgroups.

Figure 3A.

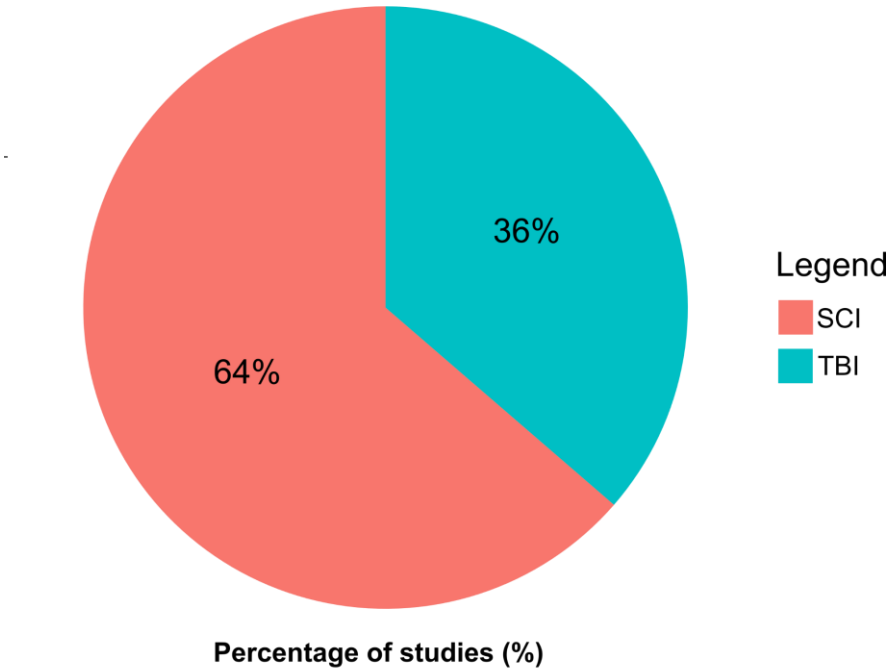

Figure 3A: Distribution of subject neurotraumatic injury types within studies:

Indicates the percentage of studies who investigated spinal cord injury (SCI) or traumatic brain injury (TBI) as the main neurotraumatic lesion.

Figure 3B.

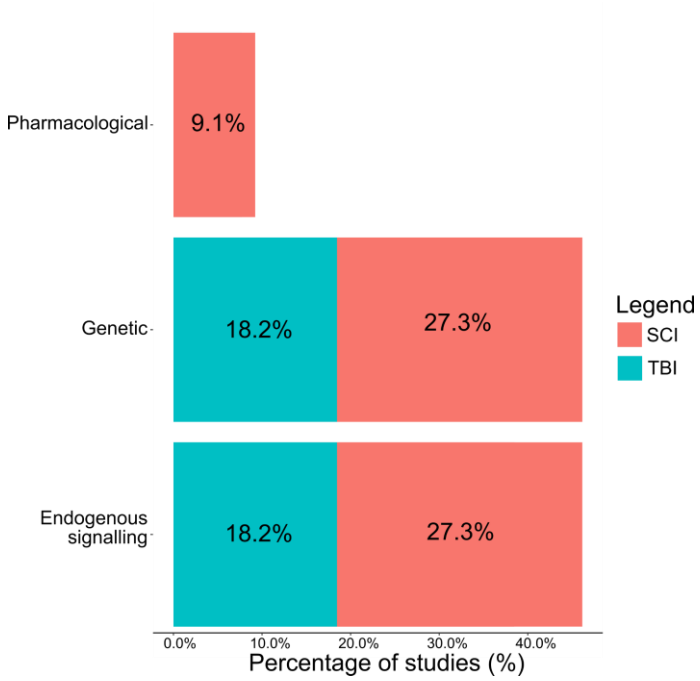

Figure 3B: NG2-glia neurogenesis strategy:
Studies were stratified according to how NG2-glia neurogenesis was attempted using either pharmacological agents, genetic modifications, or through the endogenous signaling of the injured CNS. Indicates the percentage of studies using each methodological stratus to investigate NG2 neurogenesis in spinal cord injury (SCI) or traumatic brain injury (TBI) animals.

In regard to the general description of current literature, we found that all studies were performed exclusively in rodents, with the majority of studies investigating NG2 neurogenesis in SCI at rats, as indicated in Figure 3C. Considering that TBI and SCI have gender-biased functional outcomes, with reports indicating gender-dependent improved recovery and that many cells within the CNS have sexually dimorphic responses to injury and damage, such as microglia (Doran et al., 2019; Eyolfson et al., 2020; Stewart et al., 2020), we found intriguing that the majority of studies investigating SCI models were performed in female rodents; albeit a significant number has not made clear the sex of the investigated animals, which may drastically alter this report, indicated in Figure 3D. Further, these studies are scattered homogeneously within a

timeframe of years 2006 to 2021, with a relatively low number of articles per year, which once again highlights the pioneering nature of these reports, as indicated in Figure 3E.

Figure 3C.

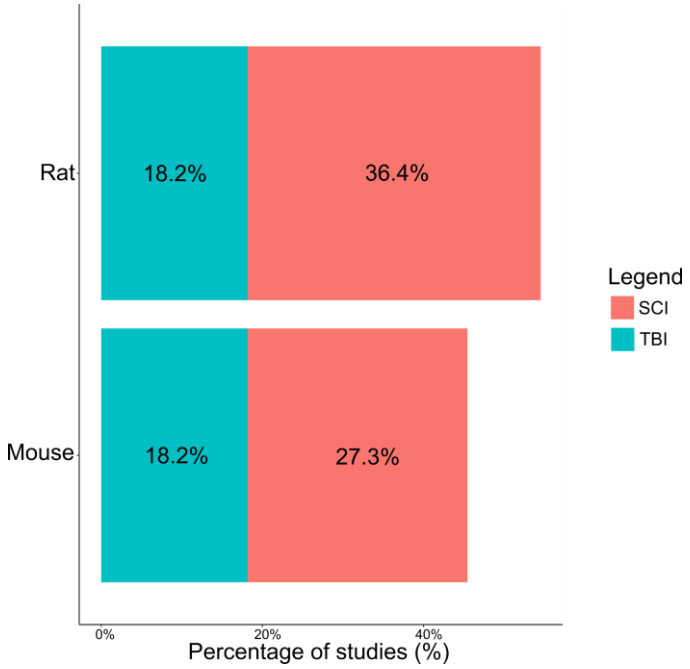

Figure 3C: Distribution of subject species within neurotraumatic injury types:

Indicates the percentage of studies investigating in rats and mice, discriminating spinal cord injury (SCI) or traumatic brain injury (TBI) as the main neurotraumatic lesion.

Figure 3D.

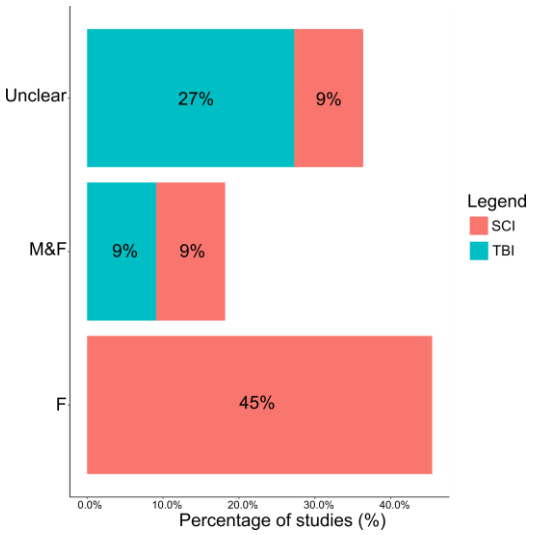

Figure 3D: Distribution of subject sex within neurotraumatic injury types:

Indicates the percentage of studies investigating female (F), male and females (M&F) as the subject sex, as well as studies where sex was not disclosed (Unclear), discriminating spinal cord injury (SCI) or traumatic brain injury (TBI) as the main neurotraumatic lesion.

Figure 3E.

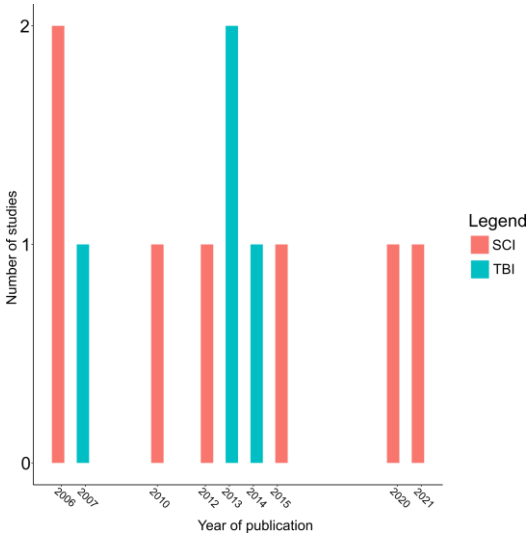

Figure 3E: Time distribution of studies within neurotraumatic injury types:

Indicates the number of yearly published articles, discriminating spinal cord injury (SCI) or traumatic brain injury (TBI) as the main neurotraumatic lesion

3.1 Protocol of NG2 neurogenesis in TBI

Preclinical TBI models are well documented and to date, robust and reliable models have been established, such as the Controlled Cortical Impact (CCI), that have allowed fundamental boosts to the scientific progress about head injuries and neuronal degenerative diseases. Along with the CCI model, various other models have been previously established and used such as the fluid percussion, and weight-drop (Marmarou) injury, that both replicate the chronic

and diffuse histological, biochemical and behavioral features of TBI in humans, in lissencephalic and gyrencephalic animals.

Out of the 4 selected papers that use TBI as the trauma model, 2 used the stab-wound injury protocol (published in 2013 and 2014), 1 used the NMDA driven injury protocol (published in 2007) and 1 used the oxygen-glucose deprivation (OGD) protocol (published in 2013). These models lack key components of TBI pathology, such as the diffuse nature in the stab wound injury, or only attempt to replicate part of the pathology such as oxygen deprivation due to loss of cerebrovascular autoregulatory function, or excitotoxicity due to glutamate vesicles release following TBI; offering models with limited construct validity when compared to more robust and modern models. Here, we will critically review their findings to summarize their results.

3.1.1 Endogenous signaling

As it was discussed before, since the discovery of NG2 cells, it was debated if they had any neurogenic potential, leading to the development of fundamental studies in animal models. After their inconclusive findings and acknowledgment of the important role that NG2 cells have in gliosis, a pioneer study (Webber et al., 2007) searched for NG2 derived neurons in brain-injured animals treated with an ex-vivo transplantation of minimally manipulated NG2 cells. That minimal manipulation is the central key of the study as it tries to reveal any innate neurogenic potential of the NG2 cell under injury.

To test that, transgenic rats with a green fluorescent protein (GFP)-NG2 cells were used as donors for GFP-NG2 cell culture as they are easy to track in future tests. These cultured cells were then transplanted into an intact young adult (8 weeks) WT rat hippocampus. 1-10 weeks after the transplantation a *N*-methyl-*D*-aspartate (NMDA) injection was performed in the animals. Excitotoxicity mediated by sustained activation of glutamatergic NMDA receptors is a known substrate of neuronal damage and loss following TBI. Although this model lacks construct validity as compared to models with actual mechanical injury, NMDA administration does recapitulate this excitotoxic component of TBI, allowing the authors to investigate the neuroregenerative

potential of NG2 cells. In fact, NMDA administration alone results in loss of dendritic spines and changes to synaptic activity (Swiatkowski et al., 2017).

Through immunohistochemistry and comparison with previous transplantations made in animals without injury, they found substantially fewer GFP+ cells and mostly differentiated to oligodendrocytes in the injured animals. These results led them to conclude that minimally manipulated populations of cortical NG2 cells should remain committed to the oligodendrocyte lineage and that endogenous signaling alone is not sufficient to turn them into a source of new neurons, so they changed their focus to other cells.

Assuming that glia and neurons are functionally interdependent and that the cell-to-cell stimulation is desirable and highly probable in restorative cascades following various CNS insults, a co-culture of freshly isolated rat neonatal NG2 cells and ischemically injured rat hippocampus slices (which were not the same animals) to mimic *in vitro* the compromise of cerebrovascular autoregulation, and blood perfusion following TBI was made (Sypecka et al., 2013). That injury protocol consisted of temporal oxygen/glucose deprivation to the slices until it was confirmed by propidium iodide that the cells were severely injured. The main goal was to evaluate if the cell signaling of the injured slices alone would be able to induce the freshly isolated NG2 cells to differentiate into neurons.

Neuronal and NG2 markers were investigated and quantified relative to non-injured hippocampal co-cultures and it was found that NG2 cells translocate and differentiate into new neurons under the interaction with hippocampal cells. Surprisingly, the injury significantly attenuates the degree of translocation and differentiation of NG2 cells. These results highlight the neurogenic potential of NG2 progenitor cells under physiological signaling, but importantly bring to the discussion a potential disruption of this regenerative mechanism following injury, a consequence of already known biochemical mismatches following injuries, such as mitochondrial dysfunction and sustained neuroinflammation, or be a new independent component. Altogether, these works suggest that although NG2 cells do differentiate into neurons, in consequence of TBI-associated mechanisms of excitotoxicity and metabolic imbalance (oxygen deprivation), their differentiation will prioritize non-neurological fates.

It is important to highlight that these studies employed more simplistic models of TBI, that do not replicate other important components of the injury, such as axonal pathology and especially the acute neuroinflammatory response; the consequence of the release of cytokines and damage associated patterns (DAMP's) that are crucial to microglial activation in TBI, and that are also recognized by NG2 cells. Nonetheless, even considering the limitations of the models and the potential underestimation of NG2 neurogenesis under endogenous conditions, it is evident that this response following injury is suboptimal for brain recovery, as functional recovery in injured patients is limited, and could perhaps be improved through genetically manipulated NG2 cells or pharmacological treatment.

3.1.2 Genetic reprogramming

With the advances of reprogramming techniques and our knowledge about transcription factors of the CNS, studies using genetic approaches to differentiate NG2 cells into neurons arose after the uncovering of their inability to naturally specialize as cell types other than oligodendrocytes.

Guo et al., (2014) and Heinrich et al., (2014) both took advantage of the retrovirus' characteristic of only infecting dividing cells and used it where the only dividing cells would be innate glial cells responding to brain trauma signals. Both use a stab-wound injury in mice as the trauma protocol but the first has a broader approach as they found astrocytes markers between the infected cells.

This study only used the NeuroD1 transcription factor in their retrovirus construct and changed the promoter accordingly in tests aiming to reprogram astrocytes or NG2 cells. By using the respective markers, they found that this methodology generates glutamatergic neurons from astrocytes and both glutamatergic and gabaergic neurons from NG2 cells. Retroviral infection induced neurogenesis at 8 days post-injection (DPI), indicated by an increase in DCX+ (doublecortin), NeuN+ (neuronal marker), and Tuj1+ (neuronal-specific beta-tubulin III) cells, proxy indicators of adult neurogenesis. An *in vitro* culture of mice cortex NG2 cells was made and the same retroviral transfection was

employed to perform patch-clamp recordings which showed that these newly formed neurons were able to fire action potentials. Based on their findings the authors concluded that it is possible to reprogram injury-induced reactive NG2 cells with a retrovirus expressing the NeuroD1 transcription factor into glutamatergic and gabaergic neurons that fire action potentials and properly integrate into the neuronal mesh.

Meanwhile, the latter study (Heinrich et al., 2014) used the Ascl1 and Sox2 transcription factors via two different retroviruses that were applied simultaneously to the animals after the TBI procedures. That methodology allowed cells to express both Ascl1 and Sox2.

They found that by applying both retroviruses, an increase of 30% of DCX+ cells was achieved in contrast to 1% with Ascl1 and 14% with Sox2, retroviruses alone, with many of these cells showing neuronal phenotype and incorporating the thymidine-analog bromodeoxyuridine (BrdU) at 12 DPI (BrdU is an important marker for immature neurons). This data demonstrates that Sox2 and Ascl1 induce conversion of nonneuronal cells into neurons in the injured adult murine cortex.

A morphology analysis was done by comparing the DCX+ cells of the different tests and it was found that the animals that were treated with both retroviruses had new neurons with a greater tendency of having more complex physiology.

To find out what cell origin these new neurons had, they used a bacterial artificial chromosome (BAC)- transgenic mouse line (Sox10-iCreERT2/GFP) in which GFP reporter expression can be specifically induced upon tamoxifen-mediated recombination in cells with an active Sox10 promoter and traced in their progeny, which is exclusively cells of the oligodendrocyte lineage, including NG2 glia and oligodendrocytes in the cerebral cortex. The tests indicated that the infected cells were indeed from an oligodendrocyte lineage and, hence the retroviruses only infect dividing cells, they concluded that the new neurons were, before the induction, NG2 cells.

To evaluate if these neurons could fire action potentials, some of them were randomly selected to go through a patch-clamp test. The majority of the selected cells did not fire an action potential as a response to the current injection but 58,8% showed a spontaneous inward current and a minority (11,7%) generated TTX-sensitive spike-like potential changes. A 3D imaging was made with the data collected from the previous tests and it showed GFP+ axonal varicosities wrapping around the soma and processes of induced DCX+ cells and, in line with these electrophysiological data, spine-like protrusions. These results led to the conclusion that these cells can incorporate nearby neuronal webs.

As all DCX+ cells were found near the injury area, they were uncertain if the lesion itself had any impact in the glia-to-neuron conversion, so they performed an experiment infecting the CNS with the Sox2 retrovirus but in animals that did not go through any trauma protocol and found no signal of the retrovirus infecting any cell. This result reinforces the reliability of the chosen methodology.

These studies, despite not using the same transcription factors, corroborate their results. The use of retroviruses, even though Heinrich demonstrated that no cell was infected without trauma, presents obvious clinical limitations, but has an important place as a proof-of-concept work, showing the potential of these cells. However, future genetic approaches - aiming at NG2 cells in TBI cases - that use these articles as a reference and add another step, such as ex-vivo transplantation, to avert this problem could have a huge impact on trauma suppression and in the development of new treatments for neuronal loss.

3.2. Protocol of NG2 neurogenesis in SCI

The NG2 cell is also homogenous and reactive to trauma in the spinal cord. Therefore, different approaches aiming at neuronal induction of them in SCI were done concurrently with the TBI studies. However, the difference in the selected tissue changes the possibilities of treatment protocols, with a lot of studies choosing the ex-vivo transplantation as it was successful with many

other studies involving SCI and other cells as treatment (Ahuja et al., 2017; Shao et al., 2019).

Such an approach usually consists in assembling an NG2 cell culture, performing an SCI protocol in rats or mice, and then inserting the cells from the culture into the injury site. By doing this, different tests can be done, like incubation of drugs in culture medium; waiting different periods before implanting the cells; utilizing exogenous or endogenous NG2 cells; among others.

3.2.1 Endogenous signaling

Kang et al., (2006) utilized said methodology using a neurobasal medium and rat adipose tissue-derived stromal cells (rATSCs) as the source of the NG2 cells for the cell culture. After 3 days of performing a laminectomy protocol for SCI in rats, these NG2 cells were labeled with carboxyfluorescein diacetate, succinimidyl ester (CFDA) dye, and transplanted by intravenous infusion. Four weeks later they were evaluated by the Basso, Beattie, Bresnahan (BBB) score. The BBB table showed that the treated group had a significant locomotor improvement over the untreated controls in just 10 days of tests. A comparison between the lesion sizes of treated and untreated groups showed that, at 4 weeks postoperatively, the volumes of cavities were significantly lower in the first.

Then, it was performed a detailed morphological and histological analysis. As the cells were prelabeled with CFDA, it was possible to see that around 30% of them showed in the lesion area but some were seen integrating into the brain (8%), kidney (12%), liver (7%), and lung (3%). The grafted cells displayed antigenic properties of astroglial cells (GFAP), OPCs (A2B5), oligodendrocytes (MBP), and neuronal cells (TuJ, NF160).

These results led them to conclude that the transplanted cells survived and migrated to the injury site very efficiently, partially differentiated into neurons

and oligodendrocytes. That migration helped the injured tissue to heal and improved the locomotor abilities of the animals.

Erceg et al., (2010) instead utilized human embryonic stem cells (hESC) derived NG2 cells for the culture and a complete transection as the SCI protocol. These cells were treated with 50% hESC growth media and 50% glial restriction media. After the rats reached the acute phase of SCI, Hoechst labeled NG2 cells from the culture were transplanted rostrally and caudally to the lesion site.

Transplanted cells survived and migrated over short distances of the spinal cord during the post-implantation survival period in all treated animals with most of the human nuclei positive cells being located in the lesion site. Immunohistochemical analysis showed that these cells were positive for NG2, NF70, GFAP, O4, PAX2, and APC. These findings indicate that the transplanted cells differentiate into astrocytes, oligodendrocytes, and neurons after interacting with the injured tissue.

A BBB score test was made with the animals and the resultant table shows that the treated group had significant locomotor improvement after 5 days. *In vivo* electrophysiological tests were made and demonstrated complete interruption of spinal motor pathways after the injury. In control animals, MEP - variable that they used to evaluate the function of spinal cord descending tracts (García-Alías et al., 2003) - did not recover over time, indicating that interruption of supraspinal axons was maintained until the end of the observation period. However, the MEP of the treated group reappeared 40 days after grafting.

This study was also searching for results with transplantation of motoneurons progenitors (MP) so all the previous tests were also made with groups of animals treated with NG2 cells and MPs. These groups presented the same immunohistological, BBB, and electrophysiological results as the uninjured controls.

To ensure that the functional recovery was caused by transplanted human cells, they isolated total RNA from the lesion site and converted this to cDNA 4

months after transplantation. They found the expression of human GAPDH, GFAP, NG2, TUJ1, and MAP2 but not in the control rats or non-lesioned part of the spinal cord. This confirms that the transplanted cells go through differentiation and the MAP2 expression is supporting evidence of their neurological potential.

More recently, another study (Patil et al., 2021) also used human stem cells - but in this case, it is human-induced pluripotent stem cells (iPSC) - as the source of NG2 cells for the culture. An accelerated defined neural induction protocol - previously described by them in Walsh et al., (2017) - was done to promote a neurological differentiation from these cells. They transplanted the cells into the lesion site ten weeks after a contusion SCI protocol was performed in rats. Simultaneously, another experimental animal group was assembled where, just before the trauma (9 days), an glial scar ablation (GSA) technique by the photo scar ablation - described at Zhang et al., (2007) - was performed as they hypothesized that GSA along with cell transplantation may be required as a combinatorial therapy in order to achieve functional recovery.

At 12 weeks post-injury, they found that the NG2+GSA group had a considerably smaller lesion cavity than the NG2 only group. Further, the transplanted cells from the NG2 group were MBP+ and NeuN+; whilst the NG2+GSA group was NeuN+ only.

To determine whether the transplanted cells differentiated into cells expressing MBP (myelin basic protein), it was looked for human nuclear antigen-positive cells co-labeled with MBP and they detected approximately 1% of the transplanted cells generating MBP and only in spinal cords from the NG2+GSA group. This suggests that some cells transplanted into the glial scar ablated cords (NG2+GSA) group were able to generate myelin 12 weeks after injection. However, they did not observe any transplanted cells expressing MBP in spinal cords recovered from animals where the glial scar was not ablated. These results led them to conclude that without GSA more transplanted NG2 cells differentiate into neurons but the GSA was better for the myelin sheath regeneration.

That ex-vivo transplantation of manipulated NG2 cells methodology appears to be the safest way to promote damage attenuation as it has shown high rates of survival, migration, and differentiation of said cells. The endogenous signaling of the injured tissue was not sufficient, however, to consistently promote differentiation into neuronal paths.

3.2.2 Genetic reprogramming

Meanwhile, other studies were more interested in genetic approaches to treat SCI. A study from 2006 (Ohori et al., 2006) combined growth factor (GF) treatment and genetic manipulation - retrovirus design to target NG2 cells and express neurogenin2 (Ngn2) and GFP (for labeling) - to stimulate neurogenesis by endogenous progenitors *in vivo*. The GFs consisted of recombinant human FGF2, mouse EGF, and human brain-derived neurotrophic factor (BDNF) that were premixed and co-injected with retroviruses. The SCI protocol chosen was laminectomy and complete transection of the spinal cord at the tenth thoracic (T10) level.

They separated 4 groups for the tests: the control group, one with only GFs, one with only Ngn2 viruses, and one with both GFs and Ngn2 viruses. The GF-only group showed, through immunostaining, an increased amount of Tuj1+ cells compared to the control. The Ngn2 only group showed a small but significant percentage of Ngn2 virus-infected cells became HuC/D+ and NeuN+ 7 at DPI. However, when combined with GFs, much larger fractions of Ngn2-expressing cells become HuC/D+ and NeuN+. Thus, the combination of the genetic and pharmacological approaches appeared to be the most effective.

Ju et al., (2012), differently, was based on studies that show NG2 cells with an overexpression of the epidermal growth factor receptor (EGFR) accelerating the remyelination process post-CNS injury. But, as Ju wanted the neurogenic fate from NG2 cells instead, their approach was to use an EGFR inhibitor in these cells.

Their first experiment was to put the EGFR inhibitor (PD168393) into an *in vitro* culture of rat reactive NG2 cells that were prepared following a previous study (Barres et al., 1992). Using a control group as a comparison, they saw that, after the inhibition, some NG2 cells acquired a neuron-like morphology. Immunostaining demonstrated distinct expression of NF200, MAP2, and b-tubulin III in PD168393-treated cells. RT-PCR experiments showed an up-regulation of the neuronal markers NF200, MAP2, b-tubulin III, NeuroD, synapsin, and Reelin. Afterward but still with the *in vitro* culture, they used siRNA to knock out the EGFR expression of the cells and got consistent results with their previous tests.

Departing to *in vivo* tests, they performed a contusion SCI protocol in mice, applied the EGFR inhibitor into the damaged region, and realized a BBB score test. After two weeks, the treated group showed great improvement in their locomotor activity compared with the non-treated one. Further immunocytofluorescence staining revealed that the newborn neurons were BrdU+, NeuN+, and NG2+. These results indicate that the EGFR inhibition can redirect the reactive NG2 cells into a neuronal pathway to produce neurons that can integrate and help with the SCI repair.

Furthermore, they also made an experiment where they used the EGFR inhibitor in reactive NG2 cells *in vitro* and transplanted them to the injury site of an injured rat spinal cord. As a neurogenic analysis, they searched for neuronal markers in the new neurons and found that they were MAP2+, b-tubulin III+, and NF200+. Expression of functional neuronal markers such as GABA and SMI32 in the surroundings of the lesion site was also found.

With all that, they concluded that EGFR inhibition could stimulate neurogenesis from glial cells, mainly the NG2 cells, in an SCI scenario.

Another study also used a combination of genetic and ex-vivo transplantation approaches. (Liu et al., 2015) based their hypothesis on the possibility of NG2 cells containing various subpopulations with diverse cellular features and differentiation capacities, presuming that a highly enriched subpopulation of

NG2 cells from CNS may be used as an effective source for cellular therapy of neurological diseases.

With that in mind, they treated a cell culture of rat NG2 cells with telomerase reverse transcriptase (hTERT) to turn them into immortal cells. After that, they transferred them into a multilineage differentiation medium. Further immunostaining revealed that they became NeuN+, NF200+, and NSE+, leading them to confirm that this method indeed induces a neurogenic path to the NG2 cells.

Next, they realized a weight drop SCI protocol in mice and transplanted the hTERT (now GFP labeled) treated cells in the injury site. 2 weeks later, an immunohistochemistry staining revealed that these cells were NF200+, MAP2+, O1+, GalC+, and S100β+. These findings indicated that the TNCs are capable of giving rise to neurons not only *in vitro* but also *in vivo*.

Treated animals went through the BBB score test and showed an increased locomotor improvement compared to SCI-only animals. With all that, they concluded that transplanted NG2 cells were able to migrate and enhance remyelination, neuronal regeneration and promote functional recovery of mice with SCI.

The final selected study (Tai et al., 2021) based its choice to genetically manipulate NG2 cells in the results of tracking (by labeling genetically in a tamoxifen-dependent fashion in adult Foxj1-CreERT2;Rosa-tdTomato mice) the origin of DCX+ (neuronal marker) cells that showed up after SCI and concluding that their source was NG2 cells and, also, based its choice to manipulate the Sox2 gene in said NG2 cells by encountering 94% of the DCX+ cells co-expressing Sox2.

Therefore, their methodology consisted in performing a crushing SCI protocol in mice and injecting lentiviruses to ectopically express Sox2 in the dividing cells of the injury site. 4 weeks past, they found significantly more new neurons in the area and confirmed (by the tamoxifen-dependent animals) that their origin was

NG2 cells. Then it was included in the lentivirus to express the BDNF, NOG, and p75NTR neurotrophic factors to see if that inclusion would enhance the production of new neurons and it was further confirmed by immunohistochemical staining.

To test if the SCI recovery of the treated animals would be any different from control ones, they performed a grid walking paradigm test. At 14 WPI it could be seen significant differences between the groups where the treated group presented a better recovery. After the behavioral tests, the spinal cords were histologically analyzed and it was confirmed that the new neurons had Sox2 expression and the treated animals had a decreased glial scar compared to SCI only ones. With these results, they concluded that Sox2-mediated reprogramming of NG2 glia reduces glial scarring and promotes functional recovery after SCI.

This session reinforces the potential that genetic manipulation of NG2 cells has for neuronal differentiation previously discussed in the TBI one. However, the implications of an SCI treatment are better tested with a BBB score than TBI, and given the positive results presented, a lot of the path for this approach to get clinical has already been paved.

4. FUTURE DIRECTIONS

Even though the selected studies presented a huge diversity in their methodologies, only the one that did a minimal manipulation to the cells (Webber et al., 2007) concluded that NG2 cells couldn't reach a neurological path in a TBI or SCI scenario *in vivo*. That shows how replicable this outcome can be even with different approaches and what future researchers can expect from these cells under trauma.

Even pharmacological treatments were able to promote neurological differentiation from the NG2 cells as it was seen with the EGFR inhibition (Ju et al., 2012). The EGFR inhibition successfully helped injured animals to recover from their neuronal loss with nothing more than its application.

Ex-vivo transplantation had a great survival and migration rate with NG2 cells and every manipulation that was presented (immortalization, induction mediums, growth factors) was enough for the cells to acquire a neurological fate and integrate when transplanted to a lesioned tissue (Erceg et al., 2010; Liu et al., 2015; Ohori et al., 2006; Sypecka et al., 2013).

The use of retroviruses appeared to be very efficient to target NG2 cells as these viruses only target dividing cells and Heinrich et al., (2014) also shows that, without trauma, no cells were infected. The literature used for these articles led them to utilize well-known transcription factors such as NeuroD1, Sox2, and Ngn2, which all presented very positive results at their respective tests.

In literature, most studies used astroglia as the target cell for neurogenic induction (Ahuja et al., 2017) but, with all these positive results, it becomes more appealing to assemble new methodologies tackling the neurogenic induction of NG2 cells instead. However, future clinical implementations will be unfriendly to genetic manipulations so more studies aiming at pharmacological inductions are welcome. Another promising proposal is to gather host NG2 cells and then genetically induce them with retroviruses in a safe environment (cell culture) to, after careful selection, perform ex-vivo transplantation of the successful ones for them to act in the injured tissue. This way immune acceptance problems are avoided and no unwanted cells get infected.

How well the converted cells can mature and integrate is currently dependent on their innate ability to do so. That opens the opportunity for future studies to manipulate the NG2 cells' aptness to migrate for them to search signals of unpaired neurons and use that to better integrate with the local neuronal web, hopefully suppressing the damages more directly. Glial scar ablation as a way to enhance that integration was tested just once (Patil et al., 2021) and without any other manipulation, leaving the question of whether this method could collaborate with a pharmacological or genetic manipulation. In most cases, it was observed that NG2 cells differentiate into neurons but also oligodendrocytes and the implications of the simultaneous rise of these two

cells in the injured site were not the targets of any test just yet. Complex behavioral tests have yet to be explored with the TBI approaches such as memory loss, learning abilities, and mood changes.

As these methods evolve, more glial knowledge will be gathered, and closer to the limitations of such cells can the scientific community get. The implications of said manipulations and healing potentials can be explored not just for trauma scenarios but for a gamma of brain disabilities that don't have a reversal. Hopefully, this local cell has the characteristics to be a useful clinical tool in the near future.

5. CONCLUSION

The present systematic review integrates the major findings related to the potential neurogenic of NG2 cells in TBI and SCI models. NG2 cells have a manipulable neurogenic potential that can be used to suppress the local neuronal loss caused by a traumatic brain injury or a spinal cord injury. NG2 cells are able to migrate to the lesioned tissue and differentiate into neurons that integrate into the local neuronal mesh and fire action potentials, therefore enhancing the recovery of the subjects. However, we are a long way from clinical applications as the present studies have many discrepancies and many of them use not-clinically-applicable methodologies. The knowledge about what is possible to achieve with NG2 cells manipulation is yet shallow but promising.

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Conflict of interest statement

The authors declare no conflicts of interest.

Declarations of interest

None.

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Identification	Name + DOI	Minimally manipulated	Differentiation of glia-committed NG2 cells: The	*In Vivo* Direct Reprogramming of	Sox2-Mediated Conversion of NG2 Glia	Autologous Adipose Tissue-derived Stromal Cells for	Transplanted Oligodendrocytes and	Characterization and therapeutic evaluation of	Induction of Neuronal Phenotypes from NG21	Regionally specific human pre-oligodendrocyte	Growth Factor Treatment and Genetic Manipulation	*In vivo* reprogramming of NG2 glia enables adult
	Year of publication	2007	2013	2013	2014	2006	2010	2015	2012	2020	2006	2021
	Author	Daniel J. Webber	Joanna Sypecka	Ziyuan Guo	Christophe Heinrich	Soo-Kyung Kang	Slaven Erceg	Rui Liu	Peijun Ju	Nandadevi Patil	Yasuo Ohori	Wenjiao Tai
	Institutional affiliation	Department of Clinical	NeuroRepair	Department of Biology,	Physiological Genomics,	Department of Physiology	Cellular Reprogramming	Department of Physiology	Genomics and Genetics	Department of	Division of Developmental	Department of Molecular
General	NG2 neurogenesis induction	Endogenous signaling	Cell signaling	Genetic	Genetic	Endogenous/ Cell signaling	Endogenous signaling	Endogenous/ Cell signaling	Pharmacological	Endogenous signaling/ Pharmacological	Pharmacological/ Genetic	Genetic
	NG2 protocol	Ex-vivo Transplantation	Indirect co-cultured system	Retrovirus reprogramming	Retrovirus reprogramming	Ex-vivo Transplantation/Culture in	Ex-vivo Transplantation	Cell culture/ Ex-vivo transplantation	EGFR Inhibitor	Ex-vivo transplantation/Glial scar	Growth factor/Retrovirus reprogramming	Lentivirus reprogramming
	Gene modulation	-	-	NeuroD1	Ascl1/Sox2	-	-	-	-	-	Ngn2	Sox2
Exp. 1	Protocol	Focal NMDA lesions were induced at 1 and	Co-culture of NG2 neonatal cells and	*In vivo* reprogramming of reactive glial cells into	Retrovirus medieded Ascl1 and Sox2	OPCs derived from rATCSs cultured with NB medium	OPCs derived from human embrionic stem cells were	Avaliation of immortalized NG2 cells	*In vitro* inhibition of EGFR in reactive NG2	OPC transplantation in animals after SCI	Insertion of Growth factors and control viruses	Ectopic expression of Sox2 via lentivirus in NG2
	Subject type	Rat	Rat	Mouse	Mouse	Rat	Rat	Rat	Mouse	Rat	Rat	Mouse
	Sex	-	-	Unclear	Unclear	F	F	Unclear	F	F	M&F	M&F
	Strain	Sprague-Dawley	Wistar	C57/BL6	C57BL/6J	Wistar	Wistar	Sprague-Dawley	Swiss Albino	Athymic nude	Sprague-Dawley	C57BL/6J
	Age	Adult	Neonatal	Adult	10 weeks	5 weeks	Adult	Adult	8 weeks	Adult	7-9 weeks	2 months
	Trauma type	TBI	TBI	TBI	TBI	SCI	SCI	-	SCI	SCI	SCI	SCI
	Trauma protocol	NMDA	Oxygen-glucose	Stab-injury	Stab-injury	Stab-injury	Complete transection	-	Contusion	Contusion	Complete transection	Crushing
	Trauma duration	1	1	1	1	1	1	-	1	1	1	1
	NG2 start treatment	0 days	0 days	0 days	0 days	3 days	0 days	-	0 days	10 weeks	0 days	0 days
	NG2 latency	Unsuccessful	5 days	3 days	12 weeks	4 weeks	5 weeks	6 days	3 days	2 weeks	3 days	4 weeks
	Outcome accessed 1	Immunohistochemistr	Immunohistochemistry	Immunohistochemistry	Immunohistochemistry	BBB score	Immunohistochemical	Immunohistochemistry	Immunocytofluorescent	Cavity volume	Immunostaining	Immunohistochemistry
	n 1	Unclear	-	5 animals	3 animals	40 animals	14 animals	-	-	5 animals	4 animals	10 animals
	Main results 1	NG2+ PDGFRα+	Comparison of progenitor differentiation in co-	DCX+	DCX+ BrdU+	Significant improvement of the treated group was	NG2+ NF70+ GFAP+ O4+ PAX2+ APC+	NeuN+ NF200+ NSE+ MAP2+	NF200+ MAP2+ β-tubulinIII+	Cavity without considerable difference in	NeuN+ ↑Tuj1+ ↑HuC/D+ GFAP+ GalC+	DCX+
	Outcome accessed 2		BDNF neutralization	Immunohistochemistry	Morphological analysis	Lesion size measurements	BBB score	Scanning electron	RT-PCR	Immunohistochemistry	-	-
	n 2		-	5 animals	442 cells from 3 mice	6 animals	14 animals	-	-	5 animals	-	-
	Main results 2		Significantly slowed down the progenitor	=DCX+ NeuN+	Sox2 and Ascl1 coexpressing DCX+ cells	At 4 weeks postoperatively, the	Significant improvement of the treated group was	Cells exhibited a small spindle cell body with	NF200+ MAP2+ β-tubulinIII+ NeuroD+	GFAP+ NeuN+ MBP+	-	-
	Outcome accessed 3		BDNF insertion	Immunohistochemistry	Transient expression	Immunocytochemical	*In vivo* electrophysiology	-	-	-	-	-
	n 3		-	5 animals	457 cells from 3 mice	Unclear	14 animals	-	-	-	-	-
	Main results 3		Helped the NG2 cells differentiate into	=DCX+ ↑NeuN+	DCX+ cells derived from NG2 glia.	GFAP+ A2B5+ MBP+ TuJ+ NF160+	MEP reappeared 40 days after grafting	-	-	-	-	-
	Outcome accessed 4			Cortical slice recordings	Patch-clamp recordings	-	-	-	-	-	-	-
	n 4			5 animals	10 animals	-	-	-	-	-	-	-
	Main results 4			The NeuroD1-converted neurons showed large	Some cells generated TTX-sensitive spike like	-	-	-	-	-	-	-
	Outcome accessed 5			-	3d imaging	-	-	-	-	-	-	-
	n 5			-	-	-	-	-	-	-	-	-
	Main results 5			-	Spine-like protusions was seen in some DCX+ cells,	-	-	-	-	-	-	-
Exp. 2	Protocol			In vivo reprogramming of reactive glial cells into		Co-culture of the injured spinal cord tissue section	OPCs and MPs derived from human embrionic	Spinal cord injury (SCI) and cell transplantation	*In vitro* knockout of EGFR expression via siRNA in	OPC transplantation in animals after SCI and scar	Insertion of Growth factors and Ngn2 viruses	Ectopic expression of Sox2 via p75NTR included
	Subject type			Mouse		Rat	Rat	Mouse	Mouse	Rat	Rat	Mouse
	Sex			Unclear		F	F	F	F	F	M&F	M&F
	Strain			C57/BL6		Wistar	Wistar	RIII/Sa	Swiss Albino	Athymic nude	Sprague-Dawley	C57BL/6J
	Age			Adult		5 week	Adult	Adult	8 weeks	Adult	7-9 weeks	2 months
	Trauma type			TBI		SCI	SCI	SCI	SCI	SCI	SCI	SCI
	Trauma protocol			Stab-injury		Stab-injury	Complete transection	Weight-drop	Contusion	Contusion	Complete transection	Crushing
	Trauma duration			1		1	1	1	1	1	1	1
	NG2 start treatment			0 days		3 days	0 days	0 days	0 days	10 weeks	0 days	0 days
	NG2 latency			8 days		2 days	5 weeks	5 days	3 days	2 weeks	7 days	4 weeks
	Outcome accessed 1			Immunohistochemistry		Immunocytochemical	Immunohistochemical	Immunohistochemistry	Immunocytofluorescent	Cavity volume	Immunostaining	Immunohistochemistry
	n 1			8 animals		15 aggregates	14 animals	16	-	5 animals	4 animals	3-6 animals
	Main results 1			NeuN+ Tuj1+		Engrafted rATSCs and some chemotaxic factor	NG2+ NF70+	Cells didn't show any neuroglial marker.	NF200+ β-tubulinIII+	Cavity with considerable difference in size than	↑NeuN+ ↑HuC/D+	BrdU+ ↑NeuN+
	Outcome accessed 2			-			RT-PCR	Immunohistochemistry	-	Immunohistochemistry	-	Confocal images of rabies-
	n 2			-			Unclear	16	-	5 animals	-	(3-6 slices) x4 animals
	Main results 2			-			GAPDH+ GFAP+ NG2+ TUJ1+ MAP2+	NF200+ MAP2+	-	GFAP+ NeuN+	-	NG2 glia-derived neurons can make monosynaptic
	Outcome accessed 3			-			BBB score	Immunohistochemistry	-		-	-
	n 3			-			14 animals	16	-		-	-
	Main results 3			-			Significant improvement of the treated group was	=NF200+ =MAP2+ O1+ GalC+ S100β+	-		-	-

	Outcome accessed 4	-			*In vivo* electrophysiology	BBB score	-		-	-	
	n 4	-			14 animals	16	-		-	-	
	Main results 4	-			MEP reappeared 40 days after grafting	Transplanted hTERT-NG2 cells promote functional	-		-	-	
Exp. 3	Protocol		*In vitro* reprogramming of reactive glial cells into				*In vivo* implantation of the EGFR inhibitor in the		Insertion of Growth factors and Ngn2-BDNF	Grid walking paradigm	
	Subject type		Mouse				Mouse		Rat	Mouse	
	Sex		Unclear				F		M&F	M&F	
	Strain		C57/BL6				Swiss Albino		Sprague-Dawley	C57BL/6J	
	Age		Adult				8 weeks		7-9 weeks	2 months	
	Trauma type		-				SCI		SCI	SCI	
	Trauma protocol		-				Contusion		Complete transection	Crushing	
	Trauma duration		-				1		1	1	
	NG2 start treatment		0 days				0 days		0 days	0 days	
	NG2 latency		7 days				2 weeks		7 days	14 weeks	
	Outcome accessed 1		Immunohistochemistry				BBB score		Immunostaining	Grid walking paradigm	
	n 1		-				Unclear		3 animals	(12-13 animals) x3 groups	
	Main results 1		NeuN+ VGlut1+ GAD67+				The locomotor activity of animals treated was		=NeuN+ =HuC/D+ ↑Tuj1+	Reprogramming of NG2 glia leads to functional	
	Outcome accessed 2		Patch-clamp recordings				BrdU tracing		Quantification of cells	Histological analysis after	
	n 2		-				Unclear		3 animals	Unclear	
	Main results 2		NG2-converted neurons generated after NeuroD1				Inhibition of EGFR could induce neurogenesis post		Animals treated with Ngn2-BDNF viruses had a	4000 new neurons and great reduction of the	
Exp. 4	Protocol						In vivo transplantation of treated NG2+ cells into				
	Subject type						Mouse				
	Sex						F				
	Strain						Swiss Albino				
	Age						8 weeks				
	Trauma type						SCI				
	Trauma protocol						Contusion				
	Trauma duration						1				
	NG2 start treatment						0 days				
	NG2 latency						4 weeks				
	Outcome accessed 1						Immunocytofluorescent				
	n 1						Unclear				
	Main results 1						MAP2+ b-tubulin III+ NF200+/Expression of				
Conclusio	General conclusion	Unselected and unmanipulated populations of cortical OPCs remain as precursor cells, commit to the oligodendrocyte lineage and fail to respond to the extrinsic cues of a neurogenic or injured environment.	The presented work show a lineage plasticity of the NG2 progenitors, depending on the tissue local microenvironment, which might be beneficial for the strategies promoting the CNS repair based either on the endogenous cell recruitment or transplantation.	In situ reprogramming of reactive astrocytes and NG2 cells into functional neurons may offer a new approach to use internally reprogrammed neurons for brain repair.	Retrovirus-mediated expression of the transcription factors Sox2 and Ascl1, but strikingly also Sox2 alone, can induce the conversion of genetically fate-mapped NG2 glia into induced doublecortin (DCX)+ neurons in the adult mouse cerebral cortex following stab wound injury in vivo.	Transplanted rATSC-OPC cells survived and migrated into the injured region very efficiently and were partially differentiated into neurons and oligodendrocyte. Behavioral analysis revealed the locomotor functions of OPC-autografted SCI rats were significanlty restored. Efficient migration of intravenously engrafted rATSC-OPCs cells into SCI lesion suggests that SCI-induced chemotaxic factors facilitate migration of rATSC-OPCs. Engrafted rATSCs and SCI-induced chemotaxic factors indeed play an important role in proliferation, migration, and differentiation of endogeneous spinal cord-derived neural progenitor	hESC-OPC and hESC-MP, when transplanted into the spinal cord immediately after the injury, survived for at least 4 months; migrated at least 3 mm away from the lesion; differentiated into appropriate cell types without forming teratomas, and improved locomotor function.	Transplanted NG2 cells were able to migrate and enhance remyelination, neuronal regeneration and promote funcional recovery of mice with SCI.	The inhibition of EGFR signaling pathway under the gliogenic conditions could induce reactive NG2 to acquire neuronal phenotypes.	Photo-ablation treatment with the FDA approved photo toxic chemical rose Bengal was successful in removing the glial scar in a model of chronic SCI and created a microenvironment that influenced the fate of cells transplanted into the white matter surrounding the injury site. Human iPSC derived pre-OPCs were shown to be multipotent and were more likely to differentiate into cells of the oligodendrocyte lineage when transplanted into an injury site where the glial scar had been removed.	Direct administration of fibroblast growth factor 2 and epidermal growth factor into lesioned tissue could induce a significant fraction of treated cells to express immature neuronal markers. Moreover, retrovirus-mediated overexpression of the basic helix-loop-helix transcription factors Neurogenin2 and Mash1, together with growth factor treatment, enhanced the production and maturation of new neurons and oligodendrocytes, respectively.	Ectopic SOX2-induced neurogenesis proceeds through an expandable ASCL1+ progenitor stage and generates excitatory and inhibitory propriospinal neurons, which make synaptic connections with ascending and descending spinal pathways. Importantly, SOX2-mediated reprogramming of NG2 glia reduces glial scarring and promotes functional recovery after SCI.

Discussão

Os estudos selecionados apresentaram grande diversidade em suas metodologias neuroindutoras às células NG2, elas sendo: manipulações farmacológicas; manipulações genéticas; e manipulações por sinalizações endógenas *in vivo* e *in vitro*; todas sendo aplicadas tanto à modelos de TCE como de TRM.

Mesmo com essa grande diversidade, apenas um estudo (Webber et al., 2007) não obteve sucesso na formação de novos neurônios a partir das células NG2. Entretanto, esse estudo utilizou como metodologia uma manipulação mínima dessas células então, mesmo com o resultado negativo no tópico, ele não vai contra os demais resultados aqui presentes e sim corrobora com a ideia de que células NG2, se não manipuladas de maneira significativa, mantém sua diferenciação exclusivamente em oligodendrócitos no SNC adulto.

O único estudo que utilizou exclusivamente a metodologia farmacológica (Ju et al., 2012) possui grande impacto nessa seleção por estar mais próximo de uma futura aplicação clínica comparado com os estudos que utilizam as outras metodologias aqui presentes. Seus resultados positivos nos testes de recuperação dos animais modelo de TRM garantem a necessidade de futuros estudos com o fármaco utilizado (o inibidor do receptor de fator de crescimento epidermal “PD168393”) que realize testes regenerativos mais complexos e detalhados.

Estudos que realizaram transplantes *ex-vivo* obtiveram todos uma taxa significativa de sobrevivência, migração e integração das células NG2 transplantadas para o tecido lesionado. Esses resultados aumentam as possibilidades de técnicas que podem ser utilizadas conjuntamente em pesquisas futuras.

A utilização de retrovírus como a ferramenta de manipulação genética das células NG2 (Guo et al., 2009; Heinrich et al., 2014; Ohori et al., 2006) se demonstrou muito eficaz devido à característica de infecção dos retrovírus de atacar apenas células em divisão e, como são lesões no SNC, as únicas células

presentes que estão se multiplicando são células gliais. Heinrich et al. (2014) ainda constatou em seu estudo que, em um cenário de aplicação do retrovírus em um tecido não danificado, não há sinais de infecção de nenhuma célula. Fatores de transcrição utilizados por esses estudos foram: NeuroD1; Sox2; e Ngn2. Sox2 se demonstrou eficaz em mais de um estudo e consequentemente foi possível ter uma descrição mais detalhada do impacto de sua transcrição, porém todas as tentativas de manipulação genética aqui presentes obtiveram resultados positivos.

Células NG2 ainda são alvo de muitas pesquisas atuais devido ao quão recente os estudos voltados a elas são e, consequentemente, o quão incertas ainda são as conclusões de suas habilidades. Essa revisão demonstra grande potencial inexplorado derivado de pesquisas mais basais que utilizam metodologias ainda distantes da clínica, mas que obtiveram, em sua grande maioria, resultados positivos. Juntamente com a evolução das pesquisas nesse tópico evolui o conhecimento glial, e mais perto das reais limitações dessas células se consegue chegar. As utilidades dessas manipulações podem ser exploradas não só para cenários de trauma, mas também para várias incapacidades neurológicas que atualmente não possuem tratamentos regenerativos. Esperançosamente, essa célula demonstrará em um futuro próximo ter as características necessárias para se tornar uma boa ferramenta clínica.

Conclusão

Células NG2 podem ser manipuladas farmacologicamente e geneticamente para um estado de neurodiferenciação onde os neurônios derivados desses processos possuem a capacidade de se integrar ao tecido e disparar potenciais de ação, assim regenerando parcialmente danos teciduais causados por um cenário de neurotrauma e, consequentemente, acelerando a recuperação motora dos animais estudados. Esses resultados podem ser utilizados como base para o desenvolvimento de futuros estudos que pretendam utilizar metodologias mais próximas de aplicações clínicas, pois as aqui selecionadas

ainda possuem grandes restrições quanto às suas aplicações médicas, mesmo com resultados positivos.

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