NEURAL REGENERATION RESEARCH
www.nrronline.org

Neuronal circuitry reconstruction after stem cell therapy in damaged brain

Daniel Tornero*

Transplantation of neuronal precursors derived from human pluripotent stem cells is a promising therapy for the treatment of neurological disorders associated with neuronal loss, such us neurodegenerative diseases, brain trauma and stroke. The functional integration of grafted neurons differentiated from stem cells into the host injured neuronal circuitry has been a major challenge in cell therapy strategies for brain repair (Palma-Tortosa et al., 2021). Even though other cell types or mechanisms may provide modest clinical improvements, neuronal replacement and reconstruction of the damaged area are crucial for an optimal and long-term recovery. This process entails three important aspects (**Figure 1**): (1) the generation of specific neuronal subtypes representative of the damaged brain area, (2) the formation of functional afferent synaptic connections from the host brain to the grafted neurons, and (3) the establishment of functional efferent synaptic contacts from grafted cells to specific areas of the host brain. We previously showed that human skin-derived neural precursors, transplanted into the somatosensory cortex of rats after ischemic stroke, develop a pattern of afferent synaptic connections similar to endogenous neurons located in this area of the brain (Tornero et al., 2017), and form efferent connections with neurons of proper host brain structures (Palma-Tortosa et al., 2020). The ability of grafted cells to functionally integrate in the damaged host brain circuitry has been further demonstrated by other recent studies using animal models (Palma-Tortosa et al., 2021). Interestingly, also transplantation of human cortical neurons onto *ex vivo* organotypic cultures of adult human cortex proved the establishment of afferent and efferent synapses between host and grafted cells (Grønning Hansen et al., 2020). However, the mechanisms behind functional recovery and integration of new neurons into the brain network still present some unknown aspects that will be discussed in this article (**Figure 1**).

Which level of specificity is reached during circuitry reconstruction? To answer this question, we must consider that the regional identity of grafted neurons is crucial for the correct reconstruction of neuronal network (Michelsen et al., 2015; Palma-Tortosa et al., 2021). In this regard, cell identity determines the formation of efferent connections from grafted neurons to different host brain areas, through a process that takes few months, since maturation of human neurons is longer compared to murine ones (Linaro et al., 2019) and sometimes newly formed axons should travel long distances, e.g., from one hemisphere to the other (Palma-Tortosa et al., 2020). By contrast, the formation of extensive afferent connectivity from the host neurons, including neurons from non-adjacent brain areas, to grafted cells can be observed at early timepoints (6–8 weeks after transplantation) by using rabies virus-based monosynaptic tracing (Tornero et al., 2017; Cardoso et al., 2018). Consequently, these synaptic contacts are likely established as a reconnection after the death of the target neurons, or as a secondary branch from pre-existing axons. In this case, early afferent patterns will be determined by the location of the transplant, regardless of neuronal subtypes generated by grafted cells. Interestingly, ectopic transplantation of dopaminergic neural progenitors in the striatum of rats led to different afferent connectivity patterns as compared to what observed when the same cells were grafted into the substantia nigra (Grealish et al., 2015; Cardoso et al., 2018). Notably, systemic injection (i.e., intravenous or intra-arterial) of neural progenitors resulted in limited infiltration to the brain parenchyma and, consequently, low integration capacity. Therefore, both, transplant location and regional identity of grafted cells will determine the degree of specificity reached during circuitry reconstruction. Similarly, the maturation stage of transplanted neurons and neuronal fate determination will affect cell survival and synaptic connectivity: too mature neurons will reduce the survival, whereas too early progenitors will result in heterogeneous cell products and unspecific functional reconstruction (e.g., serotoninergic neurons generated after transplantation of human fetal neurons in parkinsonian patients).

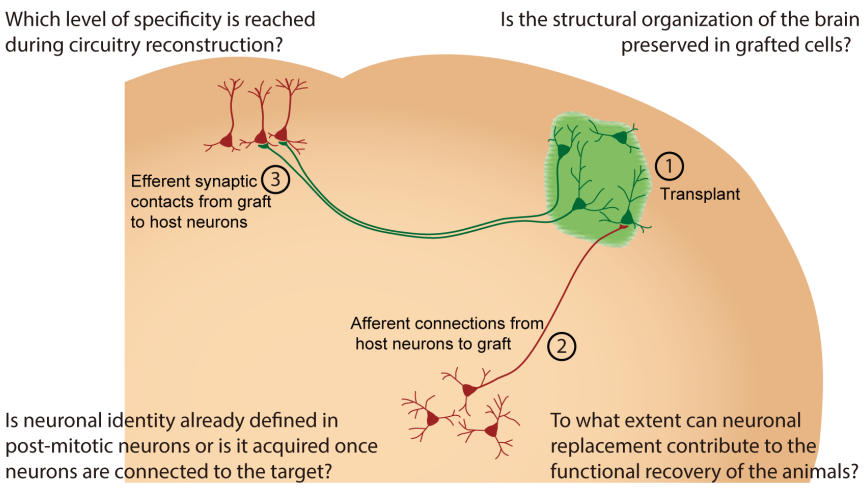

Figure 1 | **Scheme illustrating the three aspects regarding neuronal replacement (from 1 to 3) and the four questions discussed in the text (one on each corner).**
Transplanted cells are shown in green and host neurons are shown in red, in a hypothetical model of cell therapy for brain repair.

Is the structural organization of the brain preserved in grafted cells? This question is particularly important for those areas of the brain, such as the cortex, displaying a specific organization of different neural subtypes. Neurons located in a particular layer of the cortex are functionally specialized and are characterized by the expression of layer-specific transcription factors. For example, Tbr1, a transcription factor highly expressed in corticothalamic projection neurons of cerebral cortex layers 5 and 6, was 3-times more abundant in deeper areas of the grafts containing cortically fated progenitors compared to those with non-fated cells (Tornero et al., 2013). Therefore, if the organization of newly formed neurons in the adult brain is guided by local cues, cells with the proper identity will be responding to those signals more efficiently, resulting in a closer reconstitution of the cortical structure. From a functional perspective, it was demonstrated that murine fetal neural progenitors transplanted into the damaged visual cortex of adult mice integrated in the host network and showed stimulus-selective responses (Falkner et al., 2016). These grafted cells received afferent inputs from host neurons including topographically organized geniculo-cortical connections. Similar results were also obtained by using human cells (Linaro et al., 2019). However, it remains unclear how accurately newly formed neurons replicate the pre-existing spatial organization of the damaged area. In this regard, recent technologies for the analysis of whole transcriptome with spatial resolution could help answer this question. From a technical point of view, the ability to discriminate between grafted and host cells is also important. When transplanted cells are originated from the same species as recipient animal (allotransplant), the use of labelling with fluorescent reporters, PKH dyes or nanoparticles is required.

Is neuronal identity already defined in post-mitotic neurons or is it acquired once neurons are connected to the target? Recently published results, obtained by using electron microscopy and rabies virus-based monosynaptic tracing, showed that neurons derived from human progenitors grafted in a rat stroke model send projections to the contralateral hemisphere of the brain and establish synaptic contacts with host neurons (Palma-Tortosa et al., 2020). Moreover, transcallosally connected grafted cells were shown to express corticocallosal projection neuron markers, suggesting a specific

reconstruction of this pathway. However, it is unclear whether grafted neurons acquire their fate before establishing synaptic connections, or the formation of synaptic connections determines the fate of newly formed neurons. In the first scenario, committed neurons possess the information guiding the axon to the proper target; while in the second scenario, targeted elongation of axons follows common endogenous signals (e.g., white matter pathways), and once the synaptic connection is formed, the communication with the targeted neurons will determine grafted neurons fate. It is well known that the identity of the newly formed neurons is established at early stages of brain development, and is determined by a precise spatio-temporal regulation of developmental signals. However, to what extent these developmental signals are still present in the adult brain is not clear and must be further explored. Importantly, graft site microenvironment, including the type of cytokines and cellular elements of the immune response, changes from acute to chronic stages of brain damage, differently contributing to the maturation and integration of transplanted cells.

To what extent can neuronal replacement contribute to the functional recovery of the animals? The most conclusive and elegant strategy to evaluate the contribution of cell therapies to brain tissue functional recovery involve the use of optogenetics or chemogenetics (i.e., the use of designer receptors exclusively activated by designer drugs) to silence grafted neurons. To my knowledge, there are only two articles employing this strategy. In the first one, human embryonic stem cell-derived mesencephalic dopaminergic neurons expressing a light-gated chloride pump halorhodopsin were transplanted in the striatum of a Parkinson's mouse model (Steinbeck et al., 2015). Using corridor test, authors showed that transplanted animals recovered from lesion-induced Parkinsonian motor deficits, and that light-induced silencing of graft activity reversibly re-introduced lateralized behavior of the mice. Another article presents results obtained in a stroke rat model (Palma-Tortosa et al., 2020). In this work, human induced pluripotent stem cell-derived cortical neurons transplanted near the ischemic lesion affecting somatosensory and motor cortex promoted functional recovery of the animals. Optogenetic silencing of grafted neurons expressing halorhodopsin led to bilateral impairment of rats (cylinder test) 6 months after transplantation. In contrast to the results obtained with the Parkinson's model, the second study did not report, upon inhibition of neuronal activity in grafted cells, the lateralized impairment observed in the untreated group. This difference may be due to the ectopic location of the grafted cells in the Parkinson's mouse model (dopaminergic neurons are naturally located in substantia nigra while graft was placed in the striatum). This likely prevents the reconstruction of the original circuitry, and the release of dopamine in the striatum of damaged animals would only depend on graft neuronal activity. Proper location of the grafted neurons in the second study promotes the generation of specific afferent and efferent synaptic connections (Tornero et al., 2017; Palma-Tortosa et al., 2020), leading to the reconstruction of the pre-existing circuit, particularly the transcallosal communication. This may explain the bilateral impairment observed upon silencing of the grafted neurons in the rat stroke model. As shown by using intact rats injected with halorhodopsin-expressing lentivirus, silencing of cortical neurons from somatosensory and adjacent motor areas in one of the hemispheres leads to bilateral impairment (Palma-Tortosa, 2020), demonstrating that this should be the expected result when inhibiting the activity of neurons functionally integrated in this area of the brain. These two studies demonstrate the contribution of grafted neuron-derived activity in long-term functional recovery in animal models, opening new avenues for the development of clinical applications.

In summary, recent advances from studies on animal models have provided supporting evidence that new neurons can replace dead cells following injury, a critical aspect for optimal, long-term recovery of brain function. Future investigations should aim at understanding the mechanism behind the beneficial effects of stem cell therapy for brain damage repair. Despite some clear limitations of this approach (Forbes and Andrews, 2021; Palma-Tortosa et al., 2021), the clinical application of stem cell-based treatments is becoming a reality. Mutual collaboration between basic and clinical research should help overcome current limitations and future challenges of stem cell-based treatments in clinical practice.

I would like to thank Sara Palma-Tortosa for valuable discussions.

The present work was supported by Ministerio de Ciencia e Innovación (Spain), under project No. PID2020-118120RB-I00; European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 813851; and European Union's FET-Open program grant agreement, No. 9648877 (to DT).

Daniel Tornero*
Department of Biomedical Sciences, Institute of Neuroscience and Production and Validation Center of Advanced Therapies (Creatio), University of Barcelona; August Pi I Sunyer Biomedical Research Institute (IDIBAPS); Network Center for Biomedical Research in Neurodegenerative Diseases (CIBERNED), Barcelona, Spain
***Correspondence to:** Daniel Tornero, PhD, daniel.tornero@ub.edu.
https://orcid.org/0000-0002-4812-4091 (Daniel Tornero)

Date of submission: July 29, 2021
Date of decision: September 15, 2021
Date of acceptance: October 13, 2021
Date of web publication: January 25, 2022

https://doi.org/10.4103/1673-5374.335145
How to cite this article: *Tornero D (2022) Neuronal circuitry reconstruction after stem cell therapy in damaged brain. Neural Regen Res 17(9): 1959-1960.*
Availability of data and materials: *All data generated or analyzed during this study are included in this published article and its supplementary information files.*

Open peer reviewer: *Peng Li, VCU Massey Cancer Center, USA.*
Additional file: *Open peer review report 1.*

References

Cardoso T, Adler AF, Mattsson B, Hoban DB, Nolbrant S, Wahlestedt JN, Kirkeby A, Grealish S, Björklund A, Parmar M (2018) Target-specific forebrain projections and appropriate synaptic inputs of hESC-derived dopamine neurons grafted to the midbrain of parkinsonian rats. J Comp Neurol 526:2133–2146.

Falkner S, Grade S, Dimou L, Conzelmann KK, Bonhoeffer T, Götz M, Hübener M (2016) Transplanted embryonic neurons integrate into adult neocortical circuits. Nature 539:248-253.

Forbes LH, Andrews MR (2021) Advances in human stem cell therapies: Pre-clinical studies and the outlook for central nervous system regeneration. Neural Regen Res 16:614-617.

Grealish S, Heuer A, Cardoso T, Kirkeby A, Jönsson M, Johansson J, Björklund A, Jakobsson J, Parmar M (2015) Monosynaptic tracing using modified rabies virus reveals early and extensive circuit integration of human embryonic stem cell-derived neurons. Stem Cell Reports 4:975-983.

Grønning Hansen M, Laterza C, Palma-Tortosa S, Kvist G, Monni E, Tsupykov O, Tornero D, Uoshima N, Soriano J, Bengzon J, Martino G, Skibo G, Lindvall O, Kokaia Z (2020) Grafted human pluripotent stem cell-derived cortical neurons integrate into adult human cortical neural circuitry. Stem Cells Transl Med 9:1365-1377.

Linaro D, Vermaercke B, Iwata R, Ramaswamy A, Libé-Philippot B, Boubakar L, Davis BA, Wierda K, Davie K, Poovathingal S, Penttila PA, Bilheu A, de Bruyne L, Gall D, Conzelmann KK, Bonin V, Vanderhaeghen P (2019) Xenotransplanted human cortical neurons reveal species-specific development and functional integration into mouse visual circuits. Neuron 104:972-986.

Michelsen KA, Acosta-Verdugo S, Benoit-Marand M, Espuny-Camacho I, Gaspard N, Saha B, Gaillard A, Vanderhaeghen P (2015) Area-specific reestablishment of damaged circuits in the adult cerebral cortex by cortical neurons derived from mouse embryonic stem cells. Neuron 85:982-997.

Palma-Tortosa S, Coll-San Martin B, Kokaia Z, Tornero D (2021) Neuronal replacement in stem cell therapy for stroke: filling the gap. Front Cell Dev Biol 9:662636.

Palma-Tortosa S, Tornero D, Hansen MG, Monni E, Hajy M, Kartsivadze S, Aktay S, Tsupykov O, Parmar M, Deisseroth K, Skibo G, Lindvall O, Kokaia Z (2020) Activity in grafted human iPS cell-derived cortical neurons integrated in stroke-injured rat brain regulates motor behavior. Proc Natl Acad Sci U S A 117:9094-9100.

Steinbeck JA, Choi SJ, Mrejeru A, Ganat Y, Deisseroth K, Sulzer D, Mosharov EV, Studer L (2015) Optogenetics enables functional analysis of human embryonic stem cell-derived grafts in a Parkinson's disease model. Nat Biotechnol 33:204-209.

Tornero D, Tsupykov O, Granmo M, Rodriguez C, Grønning-Hansen M, Thelin J, Smozhanik E, Laterza C, Wattananit S, Ge R, Tatarishvili J, Grealish S, Brüstle O, Skibo G, Parmar M, Schouenborg J, Lindvall O, Kokaia Z (2017) Synaptic inputs from stroke-injured brain to grafted human stem cell-derived neurons activated by sensory stimuli. Brain 140:692-706.

Tornero D, Wattananit S, Madsen MG, Koch P, Wood J, Tatarishvili J, Mine Y, Ge R, Monni E, Devaraju K, Hevner RF, Brüstle O, Lindvall O, Kokaia Z (2013) Human induced pluripotent stem cell-derived cortical neurons integrate in stroke-injured cortex and improve functional recovery. Brain 136:3561-3577.

P-Reviewer: Li P; C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y