

Primary retinal ganglion cells for neuron replacement therapy

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Comment on: Venugopalan P, Wang Y, Nguyen T, *et al.* Transplanted neurons integrate into adult retinas and respond to light. *Nat Commun* 2016;7:10472.

Abstract: Optic nerve damage as a result of trauma, ischemia, glaucoma or other forms of optic neuropathy disease, leads to disconnection between the eye and brain and death of retinal ganglion cells (RGCs), causing permanent loss of vision. Therapeutic options for treating optic neuropathy are limited and represent a significant unmet medical need. Development of a regenerative strategy for replacement of lost RGCs lies at the core of the future cell-based therapy for these conditions. Successful long-term restoration of visual function depends on the type of cells for transplantation. Primary RGCs of neonatal mice are now reported to have the potential for serving such a purpose.

Keywords: Retinal ganglion cells (RGCs); optic nerve; cell-based therapy; optic neuropathy

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Neurons from mammalian central nerve system (CNS) cannot be repaired or replaced after injury or disease. Scientists and physicians turn to regenerative medicine, by transplanting stem cells or progenitor cells into the injured nervous system to promote functional recovery. Optic nerve, the second of twelve paired cranial nerves, transmits visual information from the retina to the brain. As part of the CNS, diseases afflicted the optic nerve, such as glaucoma and other optic neuropathies, lead to permanent loss of vision or blindness due to the degeneration of retinal ganglion cells (RGCs). Stem cell and cell replacement therapies present potential treatments for retinal neurodegeneration; however, transplantation of neural progenitors or differentiated neurons has long been a challenge because transplanted RGCs not only must survive, but also must integrate into the mature retina and extend long axons that can reach the brain to contribute to the restoration of vision (1). In a recent report, Venugopalan *et al.* (2) provide intriguing evidence that transplanted RGCs derived from early neonatal mice

migrated, integrated, and developed functional connections with the host retina of adult animals. This finding lights up new hopes for RGC replacement therapy.

In this paper, the authors investigated whether GFP-labeled RGCs of early neonatal mice (P0–P5) could integrate into the mature retina following intravitreal transplantation in normal, uninjured recipient rats. They found that not only GFP+ RGCs survived in the host retina, with morphologic features close to endogenous RGCs, but a significant number of transplanted RGCs extended long neurites. Transplanted GFP+ RGCs displayed a polarity similar to endogenous RGCs, with axons growing toward the optic nerve and dendrites extending into the inner plexiform layer of the retina. Interestingly, injection of fewer GFP+ RGCs resulted in higher retention of donor cells. Judging by nuclei stain, 70% of GFP+ RGCs were retained, and more than 60% of surviving GFP+ RGCs extended neurites, with many reaching the optic nerve head (ONH) 1–4 weeks after transplantation. Moreover, about 80% of the retained GFP+ RGCs had dendrite-like

processes, displaying a variety of subtype cell morphologies. The data suggest that differentiated RGCs are capable of undergoing morphological change and integration in the host retina following transplantation.

Intriguingly, the group found that at least in one animal, GFP+ RGCs extended axons into the host optic nerves, crossing the contralateral optic tract at the optic chiasm, and reached their central targets in the brain, including the lateral geniculate nucleus and superior colliculus. Albeit preliminary, the data is the first to show that differentiated RGCs could potentially be used for transplantation and are capable of extending long axons that may reach the usual RGC targets in the brain. Importantly, transplanted cells developed synapses with physiological functions, as demonstrated by electrophysiological recordings. Furthermore, transplanted RGCs exhibited light responses similar to host RGCs with ON-, OFF- and ON-OFF-subtypes, although these synapses appeared to be immature, showing slower responses and greater adaptation. The observed spontaneous action potential firings and synaptic activities from the transplanted cells, however, indicate that they were functionally connected with the host retina and able to communicate with surrounding neurons after transplantation.

This study reports a surprising finding suggesting that postnatal RGCs may be used in cell replacement therapy for treatment of vision loss as a result of optic nerve damage. In an ideal therapy, differentiated RGCs would be produced from induced pluripotent stem cells, which can be derived from the skin or blood cells of affected patients, removing the need for embryo-derived cells and post-transplant immunosuppression. Some studies have demonstrated successful replacement of degenerating photoreceptors with transplanted stem cells or progenitor cells. Little is reported, however, about the RGC replacement. The study by Venugopalan *et al.* (2) offers promising evidence, and their data showing the large variability of stratification of the dendritic structures by transplanted cells, as imaged under confocal microscopy, suggests either the survival, or the development/differentiation of morphologically distinct RGCs following transplantation. These data implicate a potential of transplanted primary RGCs to survive, migrate and develop functional connections with host neurons in the adult retina.

The findings presented in this paper were significant and exciting; nevertheless, it has also raised several important questions which require further detailed studies. First, the source and differentiation status of donor cells are thought

to have critical impacts on the efficiency of cell replacement therapy. Primary fetal RGCs not only may be difficult to obtain clinically, but likely comprise heterogeneous populations of neurons at various differentiation stages. This paper used RGCs purified from mouse pups between postnatal day 1 and 5, leaving it unknown which subpopulation of transplanted RGCs survived, integrated and developed synaptic connections. The observed limited number of animals (n=15 of total 152) that migrated through the nerve fiber layer and the small number of GFP+ RGCs detected in the host retina all have implicated that these may be the behavior of a subpopulation of neonatal RGCs. While RGCs derived from inducible pluripotent stem cells (iPSCs) are likely represent a better source of donor cells than primary RGCs, future attempts to define the molecular properties of the RGC subpopulations that integrate would be most helpful.

Second, as in this paper, RGC transplantation was carried out in recipient rats with a normal retina, the therapeutic potential of RGC transplantation would need to be tested in diseased animal models with degenerating RGCs. The diseased retinal environment is thought not to be as receptive to transplanted primary neurons as in the undamaged eyes, due to induction of reactive gliosis and tissue scarring (3). Would primary RGCs repopulate the diseased retina as they did in the normal retina and would the transplantation lead to improvement of visual function? These are key questions that demand further investigations in the future.

Recent advancement in electroretinography (ERG) and mouse behavior assessment, such as optokinetic response (OKR) tests, has also made it possible to quantitatively assess visual function before and after cell transplantation in rodents. The immune-privilege nature of the retina offers a better opportunity for grafted cells to survival as well as reduces inflammation and immunorejection following transplantation, even when the recipient is from a different species, as it was shown by the authors.

To conclude, this paper offers promising evidence supporting the likelihood of a cell replacement strategy in treating diseased retinas involving RGC degeneration. Transplanted neonatal RGCs, at least a subpopulation of them, appear to be capable of surviving and integrating into host retinas, while maintaining their physiological properties. Whether the proposed therapy can be advanced to the clinic remains to be seen. In any case, the results of this study offer a premise for regenerating or replacing RGCs in adult mammals; these findings may also lay a

foundation for future development of transplantation therapy for neurodegenerative conditions in the brain.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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