Neuroregeneration using in vivo cellular reprogramming

Cellular reprogramming is an innovative technology used to artificially convert a mature cell type into a different cell type by molecular manipulation. The general concept of cellular reprogramming is to use master transcription factors to override the endogenous transcriptome profile of a given cell type with the transcriptome profile of the target cell type, thereby altering the cellular function and identity. One of the most well-known examples of cellular reprogramming is the use of four transcription factors, octamer-binding transcription factor 4 (Oct4), sex determining region Y-box 2 (Sox2), c-Myc, and Kruppel-like factor 4 (KLF4) to reprogram somatic cells into induced pluripotent stem cells, which led to the award of the Nobel Prize in physiology to Shinya Yamanaka and colleagues in 2012. Beyond induced pluripotency, cellular reprogramming has been used to convert one somatic cell type directly into another somatic lineage, yielding promising results for the in vitro conversion of fibroblasts directly into neurons, oligodendrocytes, cardiomyocytes, muscle cells, blood progenitors and hepatocytes.

Also, direct reprogramming has been successfully applied in vivo in multiple tissues, such as the heart, liver, retina, the brain and spinal cord. Importantly, in vivo reprogramming has the potential to convert endogenous cells into the cell type lost in disease or injury. This regenerative approach bypasses the complications associated with cell transplantation, including issues with immunocompatibility, cell delivery and long term survival/integration of donor cells. In vivo reprogramming strategy offers an alternative to transplantation-based therapeutic approaches and has tremendous potential for the advancement of regenerative medicine. Here we will review and summarize the current progress for using in vivo reprogramming for tissue regeneration, with particular focus on its use in neuroregeneration.

In vivo reprogramming for tissue regeneration: The feasibility of in vivo reprogramming was first demonstrated in the pancreas, using neurogenin 3 (Ngn3), pancreatic and duodenal homeobox 1 (Pdx1) and v-maf musculoaponeurotic fibrosarcoma oncogene family, protein A (Mafa) to reprogram pancreatic exocrine cells to functional insulin-secreting β cells in adult mice (Zhou et al., 2008). Since then, subsequent studies by others have refined the reprogramming techniques, by targeting different endogenous cell types (e.g., liver cells, intestinal cells) to generate different islet cell subtypes. Importantly, Banga et al. (2012) demonstrated that in vivo reprogramming to regenerate β cells can attenuate diabetic phenotypes in mice, thus demonstrating the therapeutic potential of this approach.

Similarly in the heart, cardiac fibroblasts are an appealing avenue for in vivo reprogramming because of their abundance and their ability to become activated and recruited to damaged sites of the heart. A previous study has demonstrated the feasibility of in vivo reprogramming of cardiac fibroblasts into cardiomyocyte-like cells, by viral delivery of the master regulators Gata4, myocyte enhancer factor 2c (Mef2c) and T-box transcription factor 5 (Tbx5) into the heart of mice (Qian et al., 2012). These transcription factors allowed the activation of cardiac gene regulatory networks, yielding successful cardiac reprogramming with a reported efficiency as high as 10–15% (Qian et al., 2012). Notably, in vivo reprogramming was reported to attenuate cardiac dysfunction and decrease infarct size in the injured heart, providing a novel strategy to promote cardiac regeneration (Qian et al., 2012).

Neural regeneration in the central nervous system (CNS) using cellular reprogramming: In vivo reprogramming in the nervous system has advanced rapidly since 2013. Earlier approaches for neural regeneration mainly focus on targeting neural stem cells to differentiate and generate de novo neurons, which recapitulates native processes that takes place during brain development. Direct reprogramming, however, targets terminally differentiated cells that can only convert into a different cell type under a coerced change.

Glia cells are the most abundant cell type in the adult brain, which represented an attractive therapeutic target for repairing injured or diseased brain. Many studies have demonstrated that glial cells can be converted into neurons in the brain and spinal cord. The first proof-of-principle of direct neuronal conversion in vivo was demonstrated by Torper et al. (2013), using Brn2, Ascl1, and Myt1l. Using a Cre mouse model, specific overexpression of these reprogramming factors in parenchymal astrocytes allows successful reprogramming to NeuN positive neurons in the striatum.

Further research has simplified the reprogramming cocktail and demonstrated that Sox2, a master regulator for neural stem cells, on its own is sufficient to induce neuronal reprogramming in vivo. Overexpression of Sox2 alone can reprogram resident astrocytes into DCX positive neuroblasts in the striatum, which can mature into functional neurons in some cases (Niu et al., 2013). Similarly, Heinrich et al. (2014) showed that Sox2 alone can induce glia to neuronal conversion in the adult mouse cerebral cortex post injury. Synaptic connection was also established between the reprogrammed neurons to their neighbouring neurons. However, such reprogramming was not observed in the absence of cortex injury, suggesting striatum and cortical glia possess differences in plasticity for reprogramming.

Overexpression of another single transcription factor, NeuroD1, in mouse models of brain injury and Alzheimer's disease can also reprogram cortical astrocytes into glutamatergic and GABAergic neurons (Guo et al., 2014). In particular, this study demonstrated that in vivo reprogramming is more efficient in reactive glial cells, such as those found in the setting of injury or diseased states. Thus, in vivo reprogramming may be used as a strategy to reduce reactive gliosis, which is widely associated with nerve injury and neurodegenerative disorders.

Although there has been exciting progress in reprogramming CNS neurons in vivo, more studies are needed to better characterize the functionality of the reprogrammed neurons and the effect of neuroregeneration in disease and injury. More detailed analysis of integration of the reprogrammed neurons into existing neural circuits would be helpful to understand how newly formed neural circuits can contribute to functional restoration of the CNS.

Reprogramming Müller glia for retinal regeneration: In the eye, Müller glia are retinal glial cells that play a critical role in providing retinal integrity and homeostasis. In response to injury and disease, Müller glia become activated and display altered morphology and physiology, resulting in reactive gliosis. Studies in a number of species indicated that Müller glia represent a cellular source of new neurons (Jadhav et al., 2009). For instance, teleost fish possesses remarkable retinal regeneration capacity. Upon retinal injury, quiescent Müller glia can dedifferentiate into multipotent progenitors and give rise to all retinal neuronal subtypes, resulting in retinal regeneration and restoration of vision. In postnatal chicks, retinal injury also causes Müller glial proliferation and neuronal regeneration, albeit with less regenerative capacity compared to that seen in teleost fish.

In contrast, acute injury to the rodent retina can stimulate Müller glia to proliferate and produce a very small number of neurons, but they are not sufficient to contribute to vision restoration in vivo (Jadhav et al., 2009). Similarly, primary culture of rodent and human Müller glia in vitro have neural stem cell characteristics and can generate retinal neurons, including photoreceptors. This supports the notion that given the appropriate stimuli, human Müller glia may potentially be directed to generate neurons as a regenerative response to repair retinal damage.

Inducing changes in cell fate to restore functional cell types provides a promising therapy for visual loss. In particular, in vivo reprogramming can be utilized to convert endogenous mammalian Müller glia into photoreceptors, thereby providing a regenerative therapy for diseases characterized by photoreceptor degeneration such as retinitis pigmentosa. Previous studies have demonstrated the feasibility and therapeutic potential of in vivo reprogramming to regenerate photoreceptors. An elegant study by Tom Reh’s group have demonstrated that overexpression of Ascl1 alone is sufficient to reprogram Müller glia in vivo to a neuronal fate in the injured retina, producing fully integrated bipolar cells, amacrine cells and photoreceptors (Ueki et al., 2015). Also, this reprogramming response seemed to be more pronounced in young mice compared to adult mice, suggesting an age-dependent difference in the permiss-
siveness for Müller glia reprogramming. Further study to assess the effect of Müller glia reprogramming in restoration of visual function in in vivo models would be very interesting. In another study, Sanges et al. (2016) reported that Müller glia can be reprogrammed in vivo, by spontaneous cellular fusion with transplanted hematopoietic stem cells, which can further differentiate into photoreceptors. Notably, this reprogramming approach to regenerate photoreceptors rescued the retinal function in a mouse model for retinitis pigmentosa. Therefore, strong support for using this strategy to treat retinal degeneration. Further studies to determine the precise reprogramming factors that allow Müller glia reprogramming would be helpful in simplifying this reprogramming strategy.

Current challenges and future directions: Compared to cell transplantation therapy, a key advantage of in vivo reprogramming is that this strategy generates new neurons from endogenous cells which are compatible with the host and already integrated with the tissue, obviating issues such as immunorejection and transplant mechanisms. However, many challenges remain for translation of in vivo reprogramming to the clinic. In many cases, the reprogramming efficiency remains low. In this regards, overcoming the epigenetic barrier during cellular reprogramming is a key challenge for the field. Also, the oxidative stress caused by the change in redox state during cell fate conversion can potentially trigger cell apoptosis. Even though cellular reprogramming has been demonstrated in many tissues using combinations of lineage-restricted regulatory factors, in-depth knowledge of cell fate-determining gene networks are needed to improve reprogramming efficiency.

The difficulty of delivering multiple reprogramming factors remains a major challenge within in vivo reprogramming strategies, which may be required for certain cell types. A promising approach to address this issue is the use of the CRISPR/Cas9 system that allows multiplex activation of endogenous genes. This was demonstrated in a recent study by Black et al. (2016), where the authors utilised dCas9-based transactivators to activate endogenous Bmn2, Ascl1, and Myt1l and directly reprogrammed mouse embryonic fibroblasts to neuronal cells in vitro. Further development to apply the CRISPR activation system in vivo would facilitate the translation for using cellular reprogramming as regenerative therapies.

Many studies have relied on viral methods to deliver the reprogramming factors in vivo. However, lentiviruses or retroviruses can randomly integrate into the genome and thus development of safer gene delivery method, such as adeno-associated viruses, to target specific cell types within the tissue would be desirable. An interesting direction would be the use of small molecules to promote cell reprogramming. Zhang et al. (2015) has demonstrated reprogramming of glial cells by using a cocktail of small molecules to convert cultured human astrocytes into neurons, including LDN193189, SB431542, TTNPB, Tzv, CHIR99021, DAPT, VPA, SAG and Purmo. The chemical reprogramming of astrocytes is achieved through both transcriptional and epigenetic regulation. Small molecules promote conversion into functional neurons by activating transcription factors such as ASCL1, NGN1/2, and NEUROD1. Developing small molecules that can be easily synthesized and administered to patients would be a pragmatic approach to in vivo reprogramming. However, the screening process to discover small molecules that can specifically transduce transcription factors can be time-consuming and expensive. Other issues related to drug toxicity and delivery should also be taken into consideration for development of in vivo reprogramming using small molecules.

Conclusion: In vivo reprogramming is an emerging field that attracts enormous interest for its therapeutic potential. This technology can be used to convert endogenous cells into the target cell types, thus providing an alternative approach for regenerative medicine that bypasses many of the major obstacles posed by transplantation. In particular, a panel of transcription factors have been demonstrated to promote glial-to-neurons reprogramming, providing a promising approach for neuroregeneration. Nevertheless, several hurdles in vivo reprogramming remain, such as delivery and improvement of reprogramming efficiency. Further improvements with direct reprogramming approaches using small molecules and CRISPR/Cas9 technologies would advance development of this technology as a novel regenerative therapy for tissue repair.

RCBW was supported by grants from the National Health and Medical Research Council (1084256), the University of Melbourne (Louisa Jean De Bretteville Bequest) and the Australian National Health Foundation. The Centre for Eye Research Australia receives operational infrastructure support from the Victorian Government.

**Tu Nguyen, Raymond Ching-Bong Wong**

Centre for Eye Research Australia, Melbourne, VIC, Australia; Ophthalmology, Department of Surgery, University of Melbourne, Melbourne, VIC, Australia

*Correspondence to*: Raymond Ching-Bong Wong, Ph.D., wongcb@unimelb.edu.au.

**orcid**: 0000-0002-8902-9455 (Raymond Ching-Bong Wong)

**Accepted**: 2017-06-19

**doi**: 10.4103/1673-5374.211182

**How to cite this article**: Nguyen T, Wong RC (2017) Neuroregeneration using in vivo cellular reprogramming. Neural Regen Res 12(7):1073-1074.

**Open access statement**: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

**Contribution agreement**: A statement of “Publishing Agreement” has been signed by an authorized author on behalf of all authors prior to publication.

**Plagiarism check**: This paper has been checked twice with duplication-checking software iThenticate.

**Peer review**: A double-blind and stringent peer review process has been performed to ensure the integrity, quality and significance of this paper.

**Open peer review report**: Reviewer: Lee Onn Ching, University of Miami School of Medicine, USA. Comments to author: The author did a great job in delivering his perspective on current state of in neuroregeneration using in vivo programming. It is simple and easy to read. The author has divided the topic into cellular, tissue and organ regeneration.

**References**

Banga A, Akinci E, Greder LV, Dutton JR, Slack JMW (2012) In vivo reprogramming of Son+ cells in the liver to insulin-secreting ducts. Proc Natl Acad Sci U S A 109:15326-15341.

Black JB, Adler AE, Wang HG, D’Ippolito AM, Hutchinson HA, Reddy TE, Pitt GS, Leong KW, Gersbach CA (2016) Targeted epigenetic remodeling of endogenous loci by CRISPR/Cas9-based transcriptional activators directly converts fibroblasts to neuronal cells. Cell Stem Cell 19:406-414.

Guo Z, Zhang L, Wu Z, Chen Y, Wang F, Chen G (2014) In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer’s disease model. Cell Stem Cell 14:188-202.

Heinrich C, Bergami M, Gascón S, Lepier A, Viganò E, Dimou L, Sattor B, Berlinger B, Götz M (2014) Sox2-mediated conversion of NG2 glia into induced neurons in the injured adult cerebral cortex. Stem Cell Rep 3:1000-1014.

Jadhav AF, Roesch K, Cepko CL (2009) Development and neurogenic potential of Müller glial cells in the vertebrate retina. Prog Retin Eye Res 28:249-262.

Wang W, Zang T, Zou Y, Fang S, Smith DK, Bachoo R, Zhang CL (2013) In vivo reprogramming of astrocytes to neuroblasts in the adult brain. Nat Cell Biol 15:1164-1175.

Qian J, Huang X, Spencer CL, Foley A, Vedantham V, Liu L, Conway SJ, Fu JD, Srivastava D (2012) In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. Nature 485:593-598.

Sanges D, Simone G, Di Vicino U, Romo N, Pinilla I, Nicolás M, Cosma MP (2016) Reprogramming Müller glia via in vivo cell fusion regenerates murine photoreceptors. J Clin Invest 126:3104-3116.

Torpé O, Plister H, Wolf DA, Peerera M, Lau S, Jakobsen J, Björklund A, Greulich S, Parma M (2013) Generation of induced neurons via direct conversion in vivo. Proc Natl Acad Sci U S A 110:7038-7043.

Ueki Y, Wilken MS, Cox KE, Chipman L, Jorstad N, Sternbergh K, Simic M, Ullom K, Nakafuku M, Reh TA (2015) Transgenic expression of the proneural transcription factor Ascl1 in Müller glia stimulates retinal regeneration in young mice. Proc Natl Acad Sci U S A 112:1317-1322.

Zhang L, Yin JG, Ye H, Ma N-X, Lee G, Chen XA, Wang Y, Lin L, Chen L, Jin P, Wu GY, Chen G (2015) Small Molecules Efficiently Reprogram Human Astroglial Cells into Functional Neurons. Cell Stem Cell 17:335-347.

Zhou Q, Brown J, Kanarek A, Raphael JL, Mitchen DA (2008) In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 455:627-632.