

Background

Traumatic brain injury (TBI) can lead to necrosis, apoptosis and autophagic cell death of neurons following by multiple events such as opening of the blood-brain or blood-spinal cord barrier, inflammation, edema, ecotoxicity, increase in free-radicals, altered cell signaling and gene expression. The microenvironment of the injured brain includes different cell types such as normal and dysfunctional neurons (surviving bodies and dead axons or synapses), denervated intact neurons, senescent cells, blood capillaries, glial scars, free-radicals, neuroinflammatory and neurotrophic factors, and the extra-cellular matrix. Thus, the repair and reconstruction of the brain tissue is the ultimate goal for the treatment of brain injury. It has been observed that after injury, the brain may be attempting to repair itself, endogenous neural stem cells (NSCs) can migrate from the subventricular zone (SVZ), dentate gyrus (DG), and striatum to the injury site where they differentiate into neurons, oligodendrocytes, and astrocytes. Axon formation is a critical step to neural connection and synaptogenesis. Axon can grow and sprout from new neurons derived from NSCs. However, the spontaneous regenerative process is not sufficient for complete reconstruction and full recovery of the neuronal function (and in general in adult brain, regeneration and migration of new neurons is limited).

Also, as part of the acute injury response, glial cells (astrocytes and microglia) migrate to the injury site where they form the reactive glial scars to repair BBB, SCB and isolate the site of injury and helps in the healing process. However, the same cells start producing inhibiting growth factors limiting axonal regeneration.

Therefore, the design of neural injury response strategy needs to promote favorable factors and simultaneously inhibit adverse factors.

Cell transplantation therapy has great potential and, following cell delivery using intravenous and intraarterial injections after TBI, significant neurological improvements were observed. However transplanting stem cells in-vivo is a complex and difficult operation: 1) these cells have low capacity of migration and tend to form clusters close to the targeted site and 2) they have to integrate into the host neuronal network and survive; overall this process of migration, differentiation and integration has yet to be fully understood.

Direct cell reprogramming, or transdifferentiation, is the conversion, using typically defined transcription factors (TFs), of a somatic cell into another without inducing pluripotency. For both techniques, the main concerns are risk of tumor formation, and spread of foreign new derived neurons in unintended sites, however cell transplantation is a more involved process with a risk of triggering an immune-rejection from the host and comes with a high-cost involved in clinical grade cell production for transplantation. Yet direct reprogramming in-vivo still faces difficulties which need to be addressed:

- 1) Careful selection of the target cell for reprogramming to avoid depletion of the cells being reprogrammed: cells to be reprogrammed need to be carefully selected as not to impair their functional role.
- 2) Identify transcription factors (TFs) which promote conversion to identified neuronal cells.
- 3) Design of a precise and safe gene delivery system.

In the study we will address each of these points.

Design of an efficient and specific strategy which promotes reprogramming of astrocytes to neuroblasts in the adult brain after TBI with a low-risk gene delivery system.

Wenze Niu et al., in their study, selected astrocytes for cell reprogramming since they are one of the most abundant non-neuronal cells in the CNS. And following a TBI, they aggregate to form glial scars which physically and chemically obstruct axonal regeneration. Among the eight TFs (SCLA1, BRN2, KLF4, MYC, MYT1L, OCT4, SOX2 and ZFP521) and the four microRNAs (miR9, miR124, miR125 and miR128), they found that SOX2, by itself, induces the most significant number of DCX⁺ cells, adult neuroblast cells (iANBs). Next, they showed that iANBs were only localized in the injected striatal regions and they were not the result of any migration of DCX⁺ cells coming from areas surrounding the injection site like the lateral ventricle. With various transgenic (Tgs) mice and an inducible reporter *Cst3* gene expression they demonstrated that the iANBs originated from the cells transduced by lentivirus under hGFAP promoter and not from IBA1⁺ microglia or N2-glia, additionally they also proved that SOX-2 induced iANBS did not originate from neurons going through dedifferentiation and becoming DCX⁺ neuroblasts. Then they investigate iANBs characteristics demonstrating that the neuroblasts derived from non-dividing astrocytes and pass through a proliferating state. Finally, after co-injecting BDNF-Nog-expressing lentivirus with SOX2-lentivirus, the researchers performed a whole-cell patch-clamp which exhibited mature neurons with full electrophysiological function. In the course of the experiments, they did not observe any tumor formations confirming that the newly converted neurons have been successfully integrated into the local neuronal network without immediate side-effects.

Following the research from Nui et al., who identified SOX2 as a critical transition factor in the conversion of resident astrocytes to neuroblasts, we will express SOX2 in astrocytes. In optogenetics fine control of specific gene transcription is achieved by genetically engineering astrocytes using lentiviral, or adenovirus to express light-sensitive proteins (opsins). When illuminating opsins with a stimulating light, they undergo a conformational change inducing a change in the membrane potential of these cells. We will use light-activated CRISPR-Cas9 effector (LACE) system engineered by Lauren Polstein and Charles Gersbach. They fused the light-inducible heterodimerizing proteins CRY2 and CIB1 from *Arabidopsis thaliana* to the VP64 transactivation domain and either the N- or C- terminus of dCas9. They used four guide RNAs (gRNAs) together to increase gene expression. We propose a slight modification of their construct: 1) the gRNAs direct the binding of dCas9 to the SOX2 promoter, 2) in presence of blue light (450 nm), CRY2 undergoes a conformational change that enables heterodimerization with CIBN, 3) VP64 colocalizes with dCas9 via CRY2-CIBN interactions and induces transcription of SOX2 protein. They experienced various levels of light-dependent activations depending on the target promoter, and we will also test these three vectors: *CIBN-dCas9-CIBN+gRNA*, *dCas9-VP64 + gRNA*, *CRY2-VP64 + CIBN-dCas9-CIBN+gRNA*. Although non-viral mRNA delivery systems have recently shown promising results with biodegradable ionizable lipid nanoparticles (LNPs) encapsulating mRNA (refs), lentivirus and adeno-associated viruses (AAV) have repetitively successfully been used to deliver CRISPR/Cas9 to modify cells both in vitro and in-vivo. DNA-based replication of these viruses can result in random integration into the host and has potential genotoxicity and oncogenesis. To address those concerns, we will use Sendai virus (SeV) encoding our CRISPR-Cas9 constructs for delivery. Sendai virus is an RNA virus with no DNA intermediate and no nuclear phase, eliminating the risk of unwanted integration¹. Lastly we use CFP protein for fluorescent in-vivo imaging and various transgenic mice with details provided in the validation section.

https://www.ncbi.nlm.nih.gov.proxy1.library.jhu.edu/books/NBK299210/#ch42_sec2

¹ In the context of a purely RNA-dependent replication, we may need to flank the gRNAs by self-cleaving ribozyme replicates