

● PERSPECTIVE

Cell-based therapies for neural replacement strategies in stroke-related neurodegeneration: neurophysiological insights into stem progenitor cell neurogenesis within a host environment

The restricted neurogenesis limits the brain ability to overcome neuronal cell death following ischemic lesion: Failure of the damaged brain to regenerate following cerebral ischemia results in functional deficits those are most often irreversible and can further deteriorate, causing mortality and severe disability, progressive memory loss and cognitive impairments, known as dementia. This is caused by massive neuronal cell death and neurotoxicity following limited blood supply to the brain. Ischemic death of brain neurons (acute or delayed after lesion) has been the etiology of vascular dementia, the second most common type of dementia after Alzheimer's disease. Multiple types of vascular dementia exist, common in cognitive decline that gradually worsens and variable in where strokes happen (subcortical dementia) or how severe lesions are (e.g., major stroke, series of small strokes or brief transient ischemic attacks). It has emerged a synergistic combination of both Alzheimer's disease and vascular disease that often comes (mixed dementia). For over the last three decades after the ischemia-induced neuronal cell death had been discovered, intense research has been focused on withdrawing or, at least, attenuating neurodegeneration to improve restoring function in experimental models of post-stroke cognitive impairments while mimicking focal ischemia, permanent or transient brain lesions, hypertension or reproducing Alzheimer's disease combined with vascular pathology [for review (Hainsworth et al., 2017)]. Long-standing perspectives that ischemic neuronal death might be potentially suspended through activation of endogenous neuroprotection stay with genomic reprogramming and/or gene activation for *de novo* protein synthesis, relied on the hypoxia-activated signaling pathways (Dirnagl et al., 2009). On the other hands, pre-clinical studies, including our own works (Kopach et al., 2016; Rybachuk et al., 2017), have reported functional impairments at the later times (weeks) post-ischemia (as in principal neurons as within network), with controversial effectiveness of pharmacological therapeutics utilized after lesion. Unfulfilled regeneration convinces experimental neuroscientists to focus on cell-based therapies for neuronal replacement strategies used to overcome massive neuronal loss in the brain tissue subjected to ischemia. The major issue, however, remains how this therapy should be utilized in order to promote beneficial effects timely, namely *via* engraftment of neural stem progenitor cells (NSPCs) or already differentiated stem cell-derived neurons into the damaged brain. From that, the other conceptual question arises regarding the regulatory role of a host environment in either NSPC neurogenesis or functional integration of the grafted stem cell-derived cells. If choose the latter, what 'proper' proportion of the entirely different phenotypes needs to be grafted to possibly succeed? More insights into this are essential when considering cell therapy while aiming at remodeling of the damaged post-stroke brain from mixed stem cell-derived cells for restoring the broken functionality of such a complex tissue as the brain.

NSPC grafts mature neuronal phenotype within a host tissue in a weeks' time: The survival rate of differentiated stem cell-derived cells grafted into the organized brain tissue has been routinely low – this suggests a strong regulatory role of the host environment in the viability of stem cells. As an alternative, grafting NSPCs could potentially promote the multipotent stem cell differentiation and subsequent maturation of the entirely different phenotypes, regulated by endogenous environment, to pave the way towards 'self-repair' of damaged tissue while engaging the endogenous mechanisms of self-renewal. However, discoveries in this field are hampered by technical challenges and controversy in clinically translatable advances. Eventually, the enormous potential of stem cell therapy has been evidenced for post-stroke recovery (Tornerio et al., 2017) while its neurophysiological basis has been much less learned thus far. Repeatedly reported common patterns of firing and synaptic activity have been recorded from the stem cell-derived neurons at the very end time-points – typically few months after transplantation – that fosters underestimation of the time-dependent neurophysio-

logical integration of differentiated cells into the host tissue. In our recent study, we have traced the time courses of maturation and integration of the NSPC-derived neurons into the host circuits, demonstrating their matured excitability and synaptogenesis (to the level of endogenous principal neurons – CA1 pyramidal neurons) within 3 weeks or less *in situ* (Kopach et al., 2018). To neurophysiological point of view, biophysical properties (Na^+/K^+ conductance, capability for high-frequency firing, characteristic excitatory transmission) make these cells physiologically credible those are capable to constitute specified relationships with other cells, providing functional impact. This also points out the accelerated rate of neuronal maturation of NSPC grafts *in situ* when compared with much longer time required to achieve similar patterns of activity by stem cell-derived neurons *in vitro* (Morgan et al., 2012; Shi et al., 2012; Telezhkin et al., 2016). For restorative neuroscience, a query remains to precisely define the timing of NSPC-derived phenotypes mature and set the appropriate activity within the damaged, pathophysiological environment. Establishing this is primary to enabling the assessment of timing for emerging beneficial effects, to design and rationale further reaching perspectives for accelerating stem cell neurogenesis while considering replacement strategies for various post-stroke brain regions (subpopulations of neurons, other neural cell types).

Post-ischemic environment regulates neurogenesis of NSPC grafts: An *in situ* modelling of cerebral ischemia provides technological advantages for feasibly exploring the ischemia-induced neuronal cell death together with managing therapeutic approaches for targeted interventions in the organized brain tissue. In translational neuroscience, this methodology benefits with a meaningful consideration of variable experimental modifications utilized to mimic ischemic insult and onset of cell therapy (depending on how severe and complex the ischemic impairments are, diverse stem cell origin, etc.). In our study of an *in situ* modelling of cerebral ischemia optimized for a delayed death of principal neurons [within 2 weeks instead of acute CA1 neuronal death (Rybachuk et al., 2017)], we have traced the functional properties of NSPC grafts over the time in the hippocampal tissue subjected to ischemia (Figure 1; Kopach et al., 2018). The post-ischemic environment influenced neurogenesis of NSPC grafts in different ways. Neuronal maturation has been delayed, both excitability and synaptogenesis, at each time points tested (for over 3 weeks in total; Figure 1). Instead, multipotent NSPC differentiation has been prompted towards the non-neuronal phenotype – we have identified the NSPC-derived glial cells as fast as within a week in the post-ischemic tissue. Such principal difference in the stem cell fate between control and post-ischemic environments implies the determinant, yet underestimated, regulatory role of the host tissue in neurogenesis of NSPCs, both differentiation profile (neuronal vs. non-neuronal) and neurophysiological maturation.

Endogenous facilitators of stem cell fate: Ischemic cell damage results in the glutamate-induced excitotoxicity followed by massive neuronal death and overwhelmed neurotoxicity. Ultimately, different signaling molecules and mediators are released those levels became dramatically heightened, overloading the extracellular matrix and activating a vast majority of signaling pathways, with an immense variety of molecular mechanisms being involved. Moreover, during neuronal cell death, necrotic, pro-apoptotic factors and mediators of inflammation activate pro-inflammatory signaling, including nuclear factor-kappa B (NF- κ B) pathway and others, pertinent to overactivated astrocytic response featuring the post-ischemic brain. Numerous signaling cascades can, thus, contribute to how the ischemia-subjected environment governs the fate of NSPC grafts. The notable regulatory roles in stem cell differentiation have been evidenced for phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/protein kinase B (Akt), Ca^{2+} -dependent Ca^{2+} /calmodulin-dependent protein kinase (CaMK)/cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB), and Wnt signaling pathways in *in vitro* and *in vivo* studies, among the involved cAMP, protein kinase C (PKC) and CaMK pathways, and phospholipase C and D signaling activation (Le Belle et al., 2011; Telezhkin et al., 2016). Therefore, pharmacological or genetic manipulations modulating presently dissecting signaling pathways, providing multiple growth facilitators, such as neurotrophins, to support intrinsic growth capacity of differentiating neurons, neutralizing high levels of endogenous reactive oxygen species (ROS) in the post-ischemic tissue can accelerate neuronal maturation of NSPC grafts. Further, the supposed beneficial effects may include neurogenesis of the resident neural stem cells of the brain for activating endogenous self-renewal. By contrast, the non-neural (glial) NSPC differentiation, prompted up in the post-ischemic tissue, assumes the ROS-mediated regulation of NSPCs. The activated Notch signaling eliciting the latent neurogenic program in astrocytes after stroke (Magnusson et al., 2014) may have a synergistic effect on the post-ischemic NSPC differentiation to astrocytes.

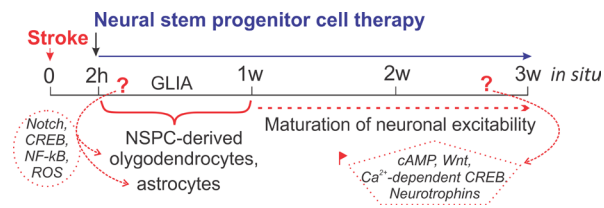


Figure 1 The experimental scheme of neural stem progenitor cell (NSPC) administration in an *in situ* model of stroke. A cartoon shows the time course for functional assessment of the NSPC-derived neural maturation in a host tissue (organotypic hippocampal tissue subjected to oxygen-glucose deprivation of 10 minutes duration, with onset of the therapy after 2 hours (h) of re-oxygenation). W: Week(s).

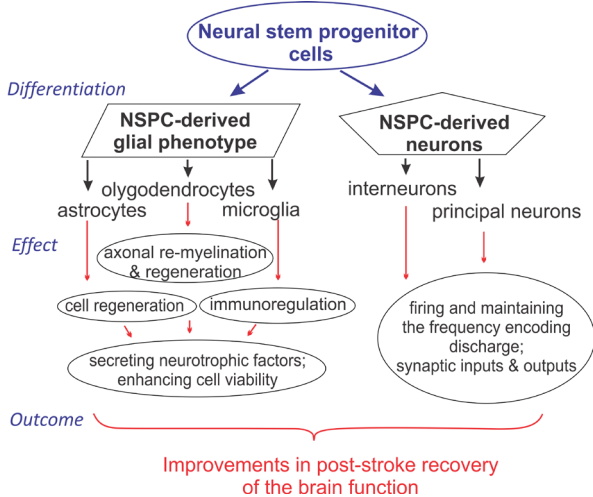


Figure 2 The current concept of NSPC-therapy in stroke-related neurodegeneration encompasses the multipotent differentiation of NSPC grafts in a host tissue for replacement of various neural phenotypes after ischemia-induced cell loss for restoring the function in multiple ways.

Functional restoration via multipotent NSPC function: Outcome after cell therapy is mediated by diverse mechanisms underlying functional improvements in the post-stroke recovery. Our study implies that NSPC therapy comprised primarily of promoted neurogenesis to a glial lineage that emerges shortly after initiating the therapy in the ischemia-subjected brain tissue (Kopach et al., 2018). This initial stage includes supplying the damaged tissue with the NSPC-derived astrocytes and oligodendrocytes – the output assuming the glia-related neurotrophic and neuroprotective effects. The stem cell-derived astrocytes enhance the viability of brain cells by secreting various neurotrophic factors and supporting cell regeneration, evidenced for improving neurological function of post-stroke brain (Jiang et al., 2013), whereas oligodendrocytes' role has been proven in re-myelination and axon regeneration, both essential to synaptic networking (Figure 2). Such glial-directed differentiation of NSPC grafts, driven by overactivated master regulators that heavily burden the post-ischemic tissue, may mirror endogenous mechanism of boosting the glia-mediated tissue clearance following ischemic neuronal cell death. As the cellular debris became cleared and pro-apoptotic and inflammatory mediators recede, multipotent NSPC neurogenesis towards neuronal maturation takes place, which is the longer lasting process. Indeed, the ability of multipotent stem cells to mature with higher efficacy to neurons in astrocyte co-cultures had been evidenced. Other roles of glial cells have included the active astrocytic contribution to synapse formation, plasticity and remodeling that should facilitate network remodeling in the post-stroke tissue.

Conclusions and future perspectives: Ischemia-induced neuronal cell death is one of the leading causes of the brain damage that results in high rate of mortality and severe disability in patients after stroke (series of strokes). The stroke-related neurodegeneration gives rise to numerous neurological disorders, including cognitive decline (vascular dementia), motor disability (paralysis), and others associated with a wide range of impairments. Massive neuronal death after stroke could be barely overcome only by pharmacological interventions, but requires also implementation of cell therapies for replacement strategies. Although the long history of stem cell research had provided conceptual advances and clinical relevance of cell-

based therapy, it has been less understood the neurophysiological basis of such tremendous effects of stem cells on the post-stroke recovery of brain function. Because recovery of the brain is not a single process, it is essential to clearly define the precise timing required for maturing of neurophysiological activity, ranging from intrinsic excitability to network function, for the entirely different stem cell-derived phenotypes. Dissecting this will expand the knowledge about stem cell therapy to find how best to use multiple routes of the therapy (induced pluripotent or embryonic stem cells) for the stroke-related neurodegeneration. More work is needed on defining the regulatory role of pathophysiological environment on stem cell neurogenesis to provide the field with a meaningful consideration of cell therapy in context of promoting 'self-repair' of the post-stroke brain by engaging endogenous brain recovery mechanisms to power self-renewal. The perspective remains in accelerating neurophysiological maturation of neuronal phenotypes in the post-ischemic tissue tailored to enhancing the functional influence and, thereby, maximizing beneficial effects. Understanding the regulatory role of pathophysiological environment on stem cell grafts will foster cell therapy use to augment therapeutic interventions in stroke-related neurodegeneration and to improve ultimate outcome.

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