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Rewiring the ischaemic brain with human-induced pluripotent stem cell-derived cortical neurons

Successful translation into the clinic of experimental molecular therapies for stroke has been limited so far, with the single exception of recombinant tissue plasminogen activator (Wahlgren *et al.*, 2007). This frustrating and rather distressing situation has in part been alleviated by the degree of spontaneous recovery that occurs in the majority of stroke survivors (Schaechter, 2004). Nonetheless, up to one-third of stroke patients remain permanently disabled and require definitive placement either in a nursing home or an assisted living environment, with great economic and social consequences (Lloyd-Jones *et al.*, 2009).

Recent advances in stem cell biology have raised expectations that diseases and injuries of the CNS may be ameliorated by the delivery of non-haematopoietic stem cell-based therapeutics. Within this context, the local versus systemic transplantation of neural stem cells has emerged as a recovery-promoting approach in preclinical models of neurological disorders, including experimental stroke (Bacigaluppi *et al.*, 2008; Martino *et al.*, 2011). Neural stem cells possess properties distinct from those of conventional therapeutics that extend far beyond the regenerative-medicine arena. Part drug and part device, transplanted neural stem cells sense diverse signals, migrate to specific sites in the body, integrate inputs to make decisions, and execute complex response behaviours, all in the context of specific tissue environments (Fischbach *et al.*, 2013).

Chronic and invalidating neurological diseases have become the ideal candidates for translating functionally flexible stem cells into clinically relevant medicines. For stroke patients, the prolonged interval between the acute onset and a delayed stem cell transplant allows the disease to stabilize, avoids first line complications and permits some degree of spontaneous recovery. A novel frontier of stem cell medicine is now arising, with focus shifting towards the idea of improving complex neurological functions (e.g. in combination with neuro-rehabilitation approaches), rather than aiming at simply reducing the size of the ischemic lesion (Dobkin, 2007).

The biggest hurdle for neural stem cell-based therapies, to date, has been the 'neuroethics' of accessing foetal or embryonic cellular/tissue sources (Ramos-Zuniga *et al.*, 2012) and the immunogenicity of the (allogeneic) graft (Aboody *et al.*, 2011). The advent of cellular reprogramming techniques has led to promising approaches for the establishment of tissue- and patient-specific human stem cell lines without the controversial use of foetuses or embryos (Liu *et al.*, 2012; Cherry and Daley, 2013). Furthermore, the most recent advances in the direct lineage conversion of somatic cells into induced neural cells *in vitro*—neurons (Vierbuchen *et al.*, 2010), astrocytes (Lujan *et al.*, 2012) or oligodendrocytes (Yang *et al.*, 2013)—provide an interesting alternative to induced pluripotent stem cell-based disease modelling.

Despite such tremendous potential, several issues arise relating to the interplay between the induced pluripotent stem cell-derived graft and the host in terms of immunogenicity, tumorigenicity and functional integration (Lindvall and Kokaia, 2011). Rigorous preclinical *in vivo* evidence is therefore needed to unravel the mechanisms behind the safety and efficacy profiles of human-induced pluripotent stem cells.

In this issue of *Brain*, Tornero and colleagues induced human pluripotent stem cells towards a functional cortical phenotype, and in so doing provided a significant step towards the clinical translation of human induced pluripotent stem cell-based therapeutics for stroke. Human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells (hiPSC-derived lt-NES; Oki *et al.*, 2012) were first fated into dorsal telencephalic cortical neurons upon *in vitro* exposure to the morphogens bone morphogenetic protein (BMP)-4 and wingless (Wnt) 3a, as well as the sonic hedgehog (Shh) inhibitor cyclopamine. Fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells displayed a significantly higher *in vitro* expression of cortical-specific markers, including the transcription factor T-box brain 1 (TBR1), the zinc finger protein CTIP2, and the homeodomain protein CDP, compared to non-fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells.

Fated and non-fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells were transplanted intraparenchymally, at two sites identified in ischaemic cerebral cortex of immune-competent (but immune suppressed) or immune-deficient (nude) rats, 48 h after experimental distal middle cerebral artery occlusion. Two months after transplantation into immune-competent (but immune suppressed) rats, fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells displayed a significantly higher differentiation into HuD-, Fox3- and

TBR1-expressing neurons, compared to non-fated cells. Interestingly enough, fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells grafts showed a 3-fold higher density of $TBR1^+$ exogenous neurons in the deep layers of the cortex, which suggests a much closer propensity to reconstitute the normal distribution pattern of endogenous $TBR1^+$ cortical neurons. A significantly higher proportion of fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells also displayed a pyramidal morphology and generated more graft-derived fibres directed to the corpus callosum, which correlated with the numbers of graft-derived $TBR1^+$ cells. Overall, fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells had lower proliferation but similar apoptotic rates, compared to non-fated stem cells.

Five months after transplantation into nude rats, fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells exhibited electrophysiological properties of mature functional neurons and monosynaptic evoked responses following electrical stimulation of the intact adjacent cortex, which suggest a host-adjusted phenotype with functional integration into the brain circuitry.

The higher integration but lower proliferation of the fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells graft—albeit not yet supported by rigorous evidence of lower teratogenic risk—suggests that the transplantation of cortically pre-differentiated human induced pluripotent stem cells is a safe and efficient option to replace the neuronal population (e.g. pyramidal neurons) that is selectively damaged after motor cortex strokes. Despite these exciting methodological and conceptual advances, the study by (Tornero *et al*., 2013) still lacks final proof of the additional value of fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells grafts on the magnitude of behavioural recovery, when compared to non-fated cellular grafts.

As suggested by the authors, prolonged times of observation shall be needed to better address assess whether the functional integration of *fated* cells would lead to further improvement as well as determining the real tumorigenic potential of both cellular grafts. Similarly, selective ablation of transplanted human cells (Cummings *et al.*, 2005) will address the correlation—if any—between the numbers of integrating cells and the extent of clinical amelioration.

Further one must consider that the observed comparable functional improvements in the two groups of middle cerebral artery occlusion rats grafted with fated versus non-fated human induced pluripotent stem cell-derived long-term expandable neuroepithelial-like stem cells, appearing at 8 weeks after transplantation, suggests that the restoration of lost functions in the injured rodent brain is largely independent from the generation of stably integrating exogenous neurons. Stem cell graft-to-host interactions lead to trophic effects on endogenous brain cells and beneficial immune modulatory actions, thus promoting the healing of the injured CNS (Pluchino and Cossetti, 2013). Nevertheless, this putative alternative (to cell integration) mechanism of tissue protection is not well understood in an experimental xenotransplantation setting, similar to that described here.

Further understanding of the multiple pathways involved in tissue rescue and functional recovery (including modulation of the host immune system, the inhibition of astroglial responses and/or the modulation of endogenous neuronal function) is needed to improve fine-tuning of the stem cell bystander activities and their neurogenic potential, eventually leading to maximum recovery (Bacigaluppi *et al.*, 2009).

As a final consideration, these data identify a more realistic window of opportunity for potential clinical application. The timing of somatic cellular reprogramming (5 weeks *in vitro*) (Oki *et al.*, 2012) and that of the subsequent neuronal fating (9 days *in vitro*) is hardly applicable to the sub-acute cellular grafting (48 h after middle cerebral artery occlusion) in ischaemic stroke patients.

This study emphasizes a novel concept of priming and retaining re-programmed cells into an expandable tissue-specific stem cell stage in order to render them more amenable for cell replacement in experimental stroke. In this context, recent advances in the generation of stably expandable directly-induced neural stem cells, circumventing the pluripotent stage, are promising (Kim *et al.*, 2011; Han *et al.*, 2012; Thier *et al.*, 2012). Future studies will be needed to further dissect the therapeutic mechanisms of induced pluripotent stem cell or induced neural stem cells grafts *in vivo*, as well as elucidate the therapeutic role of clinically relevant delayed stem cell treatments.

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