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Genetic reprogramming of somatic cells into neuroblasts through a co-induction of the doublecortin gene along the Yamanaka factors: A promising approach to model neuroregenerative disorders

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ABSTRACT

Neural stem cell (NSC) mediated adult neurogenesis represents the regenerative plasticity of the brain. The functionality of the neurogenic process appears to be operated by neuroblasts, the multipotent immature neuronal population of the adult brain. While neuroblasts have been realized to play a major role in synaptic remodeling and immunogenicity, neurodegenerative disorders have been characterized by failure in the terminal differentiation, maturation, integration and survival of newborn neuroblasts. Advancement in understanding the impaired neuroregenerative process along the neuropathological conditions has currently been limited by lack of an appropriate experimental model of neuroblasts. The genetic reprogramming of somatic cells into pluripotent state offers a potential strategy for the experimental modeling of brain disorders. Thus, the induced pluripotent stem cell (iPSC) based direct reprogramming of somatic cells into neuroblasts would represent a potential tool to understand the regenerative biology of the adult brain. Therefore, this concise article discusses the significance of iPSCs, the functional roles of neuroblasts in the adult brain and provides a research hypothesis for the direct reprogramming of somatic cells into neuroblasts through the co-induction of a potential proneurogenic marker, the doublecortin (DCX) gene along with the Yamanaka factors. The proposed cellular model of adult neurogenesis may provide us with further insights into neuropathogenesis of many neurodegenerative disorders and will provide a potential experimental platform for diagnostic, drug discovery and regenerative therapeutic strategies.

Introduction

Neurodegenerative disorders result in motor disabilities, psychiatric disturbances and dementia due to progressive degeneration of selective neuronal subpopulation in the defined regions of the brain [1]. The global incidence of neurodegenerative disorders appears to increase

Abbreviations: ASCL1, Achaete-Scute Family BHLH Transcription Factor 1; AD, Alzheimer's disease; ALS, Amyotrophic lateral sclerosis; CRISPR, clustered regularly interspaced short palindromic repeats; cMyc, myelocytomatosis viral oncogene homolog; iPSC, induced pluripotent stem cell; HD, Huntington's disease; NSC, neural stem cell; OB, olfactory bulb; PD, Parkinson's disease; RMS, rostral migratory stream; SVZ, subventricular zone; SCA, Spinocerebellar ataxia; SMA, Spinal muscular atrophy; TALEN, Transcription Activator-Like Effector-Based Nucleases; ZFNs, Zinc Finger Nucleases; PSA-NCAM, Polysialylated-neural cell adhesion molecule; Prox1, Prospero homeobox protein 1

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exponentially every year [2]. Among them, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Spinocerebellar ataxias (SCAs), Spinal muscular atrophy (SMA) and Amyotrophic lateral sclerosis (ALS) represents a great challenge to the elderly population and to the health care systems [1,3–7]. While there is an unmet need in the development of therapeutic cure for these devastating diseases, the stage-specific alterations contributing to the complex sequels of neuropathogenesis have not been fully revealed. Thus, deciphering the underlying mechanisms of neurodegenerative disorders has been the major task in the field of preclinical neuroscience. In the physiological state, neural stem cell (NSC) mediated adult neurogenesis through the generation of neuroblasts signify the self-regenerative plasticity of the adult brain [8,9]. Adult neurogenesis is an imperious cellular process of the effective maintenance of neuroplasticity that has generally been known to occur in the hippocampus and subventricular zone (SVZ)-rostral migratory stream (RMS)-olfactory bulb (OB) system of the adult brain [10–12]. Recently, occurrence of neurogenesis has also been noticed in the non-neurogenic regions such as the cortex, amygdala, hypothalamus and the striatum of the adult brain [13–16]. The generation of new neurons has been identified as an underlying cellular mechanism of cognitive functions [9,16]. It has clearly been evident that neuroblasts need to be integrated into the existing neural circuit of the brain for the replacement of degenerating neurons or for creating new connections to ensure the functional outcome of neuroplasticity including learning and memory functions [12,16]. However, the regulation of neurogenic process and mechanism for the synaptic integration of newly generated neurons in the adult brain remain unclear. Notably, the neurogenic process has been known to be regulated by various genetic, metabolic, physiological and environmental factors [16,17]. However, the neurogenic process in the hippocampus and in the SVZ appears to be differently regulated by different factors. The occurrence of hippocampal neurogenesis has been demonstrated in most adult mammalian brains including human [15,16,18,19]. However, the available reports on neurogenesis in the human brain has been a subject of debate [18,20,21]. Recently, there have been some confounding reports that suggest that the ongoing adult neurogenesis in the olfactory bulb and the hippocampus in human is highly limited [20,22]. The undetectable levels of adult neurogenesis in human have been proposed to be the result of methodological differences, chronic stress and some unknown pathogenic events [9,23]. Moreover, many neurodegenerative disorders have been characterized by impaired adult neurogenesis resulting from the quiescence of NSCs, reactive neuroblastosis, ectopic migration of neuroblasts followed by their integration failure into the existing neuronal circuit and degeneration of new neurons [15,23–27]. While reactive neurogenesis has previously been assumed to be the part of neuropathogenesis [28,29], recent reports support the idea that reactive neuroblastosis may contribute to neuroregenerative plasticity, immunogenicity and functional recovery in neurological diseases [9,15,28,30]. However, the molecular mechanisms underlying neuroblastosis and the terminal fate of reactive neuroblasts have been obscure. Indeed, neuroregenerative failure in the adult brain has been proposed to play a key role in the development of the neurocognitive syndromes including dementia [9,17,31]. Thus, understanding the functional significance and fate of reactive neuroblasts in brains of neurocognitive disorders require a great scientific attention. However, there has presently been no potential experimental model to recapitulate the molecular and cellular events of neuroblastosis and to trace the terminal fate of neuroblasts along the abnormal neurogenic process seen in neurological disease.

Emerging technological advancements in the next generation gene sequencing (NGS), omics and system biological tools have enabled the recognition of many cellular, genetic, epigenetic, metabolic and molecular abnormalities underlying human diseases. Further, the gene editing technologies like Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector-Based Nucleases (TALEN) and the clustered regularly interspaced short palindromic repeats (CRISPR) offer the scientific caliber to rectify the defective genes [32,33]. Moreover, the recent trend in the generation of disease-specific human iPSCs using genetic reprogramming of somatic cells has created a dramatic scientific insight into the neurobiology of diseases and neuroregenerative medicine [34–37]. Eventually, many human iPSC lines have been successfully created from individuals with neurodegenerative disorders [38–40]. Conversion of the patient-specific iPSCs into neurons and glia has been referred to reveal the genetic defects, differential gene expression profiling, epigenetic modifications, and cellular and molecular abnormalities underlying the neurodegenerative process [41]. Therefore, the generation of iPSCs has been considered a bona fide tool to investigate the underlying basis of brain disorders in a personalized manner [42]. However, the heterogeneous characteristics of iPSCs, improper differentiation efficiency, malignancy, trans-differentiation and dedifferentiation resulting from the genetic reprogramming, maintenance and storage have been the main limitations of modeling these brain disorders [43–45]. Despite the availability of many protocols for neuronal differentiation of iPSCs, the prolonged differentiation period, heterogeneous lineage, inadequate neuronal differentiation and their improper electrophysiological attainments tend to be the major drawbacks in understanding the neuropathogenic events [46–48]. Apparently, difficulties in achieving experimental reproducibility due to clonal variations of iPSCs have become a subject of major concern [49,50]. Thus, it demands the implication of a potent neurogenic determinant in converting the somatic cells into a dynamic, homogenous neural population and maintaining them as subcultures to monitor the disease progression.

Expression markers of neuroblasts and possible roles of doublecortin (DCX) gene in the adult brain

In the stem cell niches of the brain, the transformation of NSCs into new immature neurons has been known to be determined by the generation of neuroblasts by a process called neuroblastosis [8,9]. The migration and homeostatic regulation of neuroblasts appear to be controlled and determined by the expression of certain cellular markers [8,51–53]. The neurogenic characteristics and molecular signatures of neuroblasts has been known to be associated with the expression of the DCX gene [8,52], NeuroD [54], Achaete-Scute Family BHLH Transcription Factor 1 (Ascl1) [55], Polysialylated-neural cell adhesion molecule (PSA-NCAM) [56], Prospero homeobox protein 1 (Prox1) [57] and calretinin [58]. Among them, the expression of the DCX protein has been extensively characterized, as it represents a prominent marker for neuroblasts reflecting the ongoing neurogenic process in the adult brain [8,52,59,60]. DCX is a microtubule-associated protein that appears to be indispensable for the development of mammalian brain [61–63]. Though the expression of the DCX gene has been widespread in the migrating neuroblasts throughout the developing brain [59,62,64,65], the presence of the DCX positive neuroblasts appears to be restricted to neurogenic niches of the normal adult brain [8,52,66,67]. However, the circulation of neuroblasts in non-neurogenic niches including piriform cortex in the adult brain has also been recognized [9,15,60]. Notably, the DCX-positive neuroblasts have been shown to display a multipotent capacity similar to NSCs, thereby playing a decisive role in the steady state of neuroregenerative process in the healthy adult brain throughout the lifespan [8,66]. Importantly, the functional role of neuroblasts in the adult brain has been attributed to the motor, learning and memory functions [9,31,69,70]. The DCX positive neuroblasts have been shown to possess electrophysiological properties [70,71], thereby contributing to the synaptic remodeling of the adult brain. Recently, neuroblasts have been recognized to play a crucial role in mediating the sensorimotor inputs in the adult brain [15,60,72]. While the experimental estimates of the DCX-expressing neuroblasts have been known to positively be modulated by physical activity, enriched environment and various neuromodulators in the adult brain [73–75], the reduction in the neuroblast population has been evident in response to ageing,

chronic stress, drug abuse and neuropathologies [68,76,77]. At an early stage, many neurodegenerative disorders have been characterized by reactive neuroblastosis, a process of aberrant generation of neuroblasts and ectopic migration of neuroblasts towards the affected areas of the diseased brain [15,24,27,78–81]. However, the terminal fate of the reactive neuroblasts remains unknown in the diseased brain.

Functional relevance of reactive neuroblastosis in diseased brains

Reactive neuroblastosis has been proposed as a specific cellular trait of many neurodegenerative disorders [9]. Strikingly, reactive neuroblastosis has been reported in the early stage of brain disorders, including HD, PD, AD, ALS, stroke and epileptic seizure [15,28,82–84]. Reactive neurogenesis resulting from abnormal neuroblastosis and unusual migratory pattern of neuroblasts towards the defective brain areas has also been proposed to facilitate the brain repair and immunogenic functions [15]. Recently, reactive neuroblasts in diseased brains have been reported to express proteins thought to be specific for glial cells such as microglia and astrocytes, but the transdifferentiation of reactive neuroblasts and their neuroimmunological roles along neuropathogenesis remains unknown [9,20,85]. Thus, it can be speculated that the DCX protein may be over-expressed in reactive neuroblasts and contribute to the abnormal cell cycle events followed by an aberrant neuronal migration or trans-differentiation into glial and neuroimmune cells during the early stage of the disease. Therefore, the effect of augmentation of the DCX gene on the cell cycle regulation and fate of reactive neuroblasts needs to be investigated using an appropriate experimental system. Besides, the neurocognitive disorders that display NSC quiescence, defects in neuronal differentiation, reactive neuroblastosis and failure in their integration to the existing neural network have recently been categorized as neuroregenerative disorders [9]. Meanwhile, there are some reports suggesting that aberrant neurogenesis may contribute to the progression of neuropathogenesis [28]. In addition, a different line of argument speculates that adult neurogenesis resulting from neuroblastosis might induce some adverse effects leading to abnormal synaptic connections in the brain [16,86,87]. Therefore, the precise role of the DCX expressing neuroblasts in the healthy brain needs to be determined further in comparison with DCX cells from brains of neuroregenerative disorder subjects. Recently, many *in vivo* experimental approaches have been developed for the fate mapping of neuroblasts in the brain using a reporter-based DCX expression [88,89]. While the reporter-based DCX promoter transgenic animal models have been highly instrumental in monitoring the phenotype and migratory pattern of neuroblasts in the adult brain, these *in vivo* models may not help to determine the functional roles of the DCX gene [88,89]. Moreover, the aforementioned animal models need to be exploited for the invasive experimental procedures to mimic the neurodegenerative disorders in order to correlate the regulation of neuroblasts in the adult brain. Therefore, the establishment of the patient specific cellular model of neuroblast using an iPSC-based technique might help in providing the best alternative to understand the neurobiology of disease at the levels of biochemical, metabolic, genetic and cellular alterations.

Experimental modeling of neurodegenerative disorders using iPSCs

The existing knowledge on the pathogenesis of various neurodegenerative disorders has largely been obtained from the post-mortem brain tissues and genetically modified organisms. Despite the fact that the neuropathological alterations of the post-mortem brain tissues can only symbolize the terminal stage of illness, the impact of post-mortem delay may also affect the outcome of the results [23]. Moreover, genetic models of the brain diseases in laboratory animals may not virtually compensate the sequence of neuropathogenic events that occur in human subjects. The existing reports on adult neurogenesis in human neurodegenerative brains and the corresponding transgenic animal models and their interpretations are sometimes inconsistent, often due to methodological and technical differences [11,20,90,91]. Moreover, the available neuronal cell lines may represent molecular pathways and abnormal cell cycle events of the malignant cells due to the process of cellular immortalization and telomere abnormalities [92,93]. However, the global recognition of genetic reprogramming of the adult somatic cells into a pluripotent state offers a great opportunity to overcome the aforementioned practical disadvantages [35,43,94]. The combined induction of embryonic stem cell markers such as octamer-binding transcription factor 4 (Oct4), SRY (sex determining region Y)-box 2 (Sox2) and Kruppel-like factor 4(Klf4) along with mitotic inducer c-Myc has been shown to stimulate a pluripotent signature in the adult somatic cells [34,35]. The generation of patient-specific iPSCs has been considered as a highly promising strategy in modeling of human diseases including neurodegenerative disorders [38,95]. Recently, iPSCs have successfully been generated from human subjects of neurodegenerative disorders including HD [95], SMA [96], AD [97], PD [98], and ALS [99], and some neurodevelopmental [100] and neuropsychiatric disorders [101]. However, the current genetic reprogramming protocols that are available for the differentiation of iPSCs into neuronal phenotype appear to be highly laborious, time-consuming and expensive. Further, the data on the degree of clonal nature, lifespan and survival rate of the iPSC-derived neuronal phenotypes has been obscure [102–104]. Taken together, the existing protocols for the derivation of functional neurons from iPSCs and their subsequent maintenance in culture condition have been known to have some drawbacks. Therefore, the modeling of neurological and psychiatric disorders using iPSC-based protocols has presently been a subject of modifications with respect to neuronal differentiation, for which a suitable candidate factor that involves the neuronal differentiation, migration and electrophysiology would be highly relevant. Strikingly, iPSCs generated from lissencephaly patients have been shown to display impaired cellular differentiation, neuronal migration, and abnormal neurite outgrowth [105]. Lissencephaly is a rare neuronal migration disorder that occurs due to mutations in the DCX gene [106]. Considering the fact, the wild type DCX gene can serve as a potential candidate for the direct reprogramming of somatic cells into neuroblasts and enhance the neuronal differentiation of iPSCs. Though the data from knockout studies of the DCX gene have revealed somewhat conflicting impression [107,108], induction of the expression of the DCX gene in human iPSCs to study its gain of function will provide a potential *in vitro* model in representing the neuropathological and neurogenic pathways along the disease progression. Thus, modeling of neuroblasts using iPSCs from healthy donors and subjects of neurocognitive disorders may provide further insight into the regulation of neuroblast-mediated neuroplasticity, synaptic remodeling, the immunogenic role of neuroblasts, the terminal fate of neuroblasts and the underlying pathomechanisms of dementia.

Proposed iPSC-based model to recapitulate neuroblasts through the co-induction of the DCX gene along with Yamanaka factors

Based on the widespread existing evidence on the roles of DCX-positive neuroblasts in the neuroregenerative process and synaptic plasticity [8,9,52], the DCX gene may serve as an ideal intrinsic candidate and genetic determinant to develop a dynamic and homogeneous neural cell line with multipotential capacity. Thus, it can be hypothesized that the direct genetic reprogramming of fibroblast or peripheral blood mononuclear cells through the co-induction of full-length wild type DCX gene along with Yamanaka factors would yield a neuroblast cell line with neurogenic, immunogenic and electrophysiological properties. The co-induction of the DCX gene in somatic cells can be achieved through a suitable expression vector such as an episomal iPSC reprogramming vector [109,110]. This approach could be expected to reduce the transmission latency of neuronal differentiation and mitigate the clonal heterogeneity of the iPSC-derived neural population. Further,

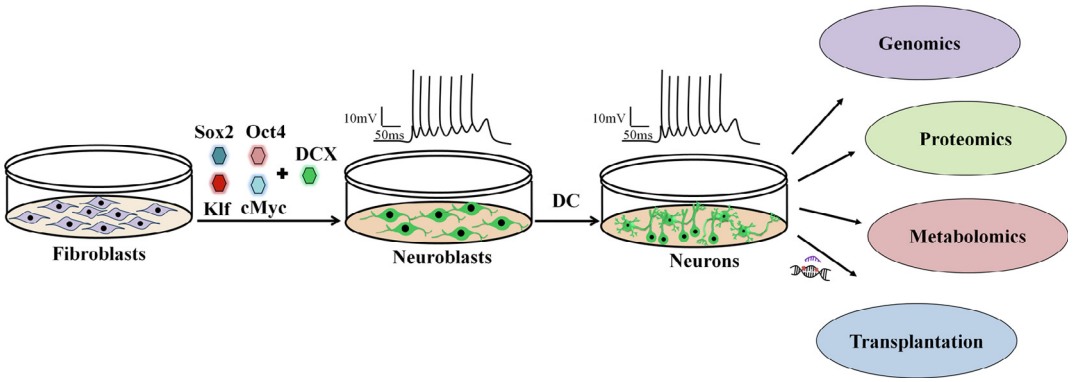

Fig. 1. The graphical representation of the proposed hypothesis on the generation of iPSC-based neuroblast cell line from fibroblasts. The illustration represents the generation of electrophysiologically positive neuroblasts and neurons from fibroblasts using iPSC technique. The figure also suggests that the generated neuroblast cells can be converted into different types of neurons using suitable differentiation condition (DC). Further, they can be implemented for various omics, gene editing and cell transplantation procedures.

the neurogenic and immunological properties of neuroblast and their terminal fate can be investigated and monitored using appropriate experimental designs in a real-time manner. The proposed model can be directly generated from the human subjects as well as from animal models with neurodegenerative disorders. Carrying out studies using human-derived iPSC-neuroblasts would greatly minimize the use of animal experiments and human embryonic stem cells which pose ethical concerns. The proposed iPSC-neuroblast model will aid in recapitulating the neuropathogenesis and neurogenesis that would be more relatable in human situations with neurodegenerative disorders. Therefore, the previous reports can be validated and a new line of experimental evidence can be generated to identify valid therapeutic targets directly using human-specific iPSC-neuroblasts. The proposed DCX-based cell line can efficiently be propagated, maintained, subcultured and converted into desired neuronal phenotype using specific differentiation conditions (Fig. 1). The iPSC based neuroblasts can additionally be implemented for the DCX promoter-based expression system or chemically labeled with tracer magnetic nanoparticles. Thus, the fate of these cells can be monitored using *in vitro*, organotypic, 3D-organoid culture and *in vivo* engraftment procedures. The cellular, genetic, genomic, proteomic and metabolomic profile of neurodegenerative diseases can be assessed during the *in vitro* differentiation of the proposed model using reporter based assays, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), NGS, protein array and advanced chromatography-mass spectrometry techniques, in which, the pathways that are related to functional neurogenesis, cell survival and cell death can be investigated in comparison with control cells. Eventually, the engraftment of the reporter-based iPSC-neuroblasts on hippocampal slice cultures from animals or human brain organoids would facilitate to monitor the fate of these cells using live imaging techniques. Eventually, the proposed cell model itself can serve as a potential tool in generating the human brain organoids to monitor the development and progression of pathogenesis and fate of neuroblasts. The reporter based iPSC-neuroblasts can be transplanted to the brains of experimental animals and the fate of these cells can be monitored using 2-photon live imaging microscope. Taken together, the iPSC based neuroblasts may represent a potential experimental model of the brain disorders that can aid in identifying the pathomechanism underlying the regenerative failure and neurodegeneration. Further, the proposed iPSC-neuroblast model would serve as a potential cellular alternate for the neural stem cell transplantation procedure (Fig. 1)

Conclusion

The progressive neurodegenerative diseases have collectively been characterized by memory loss due to neurodegeneration and failure in neuroregeneration of the adult brain. In order to visualize the underlying molecular mechanisms of neurodegeneration and to establish potential therapeutic strategies, different experimental animal and cellular models have been generated to study the pathogenic course of diseases. Among them, the emergence of patient-specific iPSCs represents a powerful tool to investigate the complexity of the human brain disorders. The reprogramming of somatic cells or directing the iPSCs into neuroblasts by the DCX gene containing vectors might provide a greater strength to the *in vitro* studies to effectively monitor the neurodegenerative and neuroregenerative processes. The proposed iPSC-neuroblasts model could serve as an alternative of neurosphere culture system, while neuroblasts can be maintained and propagated as neuronal subcultures. Eventually, this cellular model can be implemented for the high throughput screening of potential neuroprotective and proneurogenic agents. Besides, the iPSC-based DCX expressing neuroblast cell line may also serve in the eradication of the defective gene mutations using gene-editing techniques followed by autologous neural transplantation procedures. Taken together, the proposed model would facilitate in elucidating the neurogenic and immunogenic properties of neuroblasts. Moreover, the functional role of reactive neuroblastosis observed to be elicited towards the acute and progressive brain disorders might functionally be addressed using the proposed cellular model. However, unknown adverse effects of induced DCX gene expression in somatic cells related to abnormal cell cycle events or aberrant synaptogenesis may not be completely excluded.

Conflict of Interest

The authors declare there is no conflict of interest.

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References

[1] Gitler AD, Dhillon P, Shorter J. Neurodegenerative disease: models, mechanisms, and a new hope. Dis Model Mech 2017;10:499–502. https://doi.org/10.1242/dmm.030205.

[2] GBD 2015 Neurological Disorders Collaborator Group. Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol 2017;16:877–97. https://doi.org/10.1016/S1474-4422(17)30299-5.

[3] Abati E, Bresolin N, Comi GP, Corti S. Preconditioning and cellular engineering to increase the survival of transplanted neural stem cells for motor neuron disease therapy. Mol Neurobiol 2018. https://doi.org/10.1007/s12035-018-1305-4.

[4] Moily NS, Kota LN, Anjanappa RM, Venugopal S, Vaidyanathan R, Pal P, et al. Trinucleotide repeats and haplotypes at the huntingtin locus in an Indian sample overlaps with European haplogroup a. PLoS Curr 2014:6. doi: 10.1371/currents.hd.a3ad1a381ab1eed117675145318c9a80.

[5] Sathya M, Moorthi P, Premkumar P, Kandasamy M, Jayachandran KS, Anusuyadevi M. Resveratrol intervenes cholesterol- and isoprenoid-mediated amyloidogenic processing of AβPP in familial Alzheimer's disease. J Alzheimers Dis 2017;60:S3–23. https://doi.org/10.3233/JAD-161034.

[6] Velusamy T, Panneerselvam AS, Purushottam M, Anusuyadevi M, Pal PK, Jain S, et al. Protective effect of antioxidants on neuronal dysfunction and plasticity in Huntington's disease. Oxid Med Cell Longev 2017;2017:3279061. https://doi.org/10.1155/2017/3279061.

[7] Venkatesh SD, Kandasamy M, Moily NS, Vaidyanathan R, Kota LN, Adhikarla S, et al. Genetic testing for clinically suspected spinocerebellar ataxias: report from a tertiary referral centre in India. J Genet 2018;97:219–24.

[8] Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N, et al. Doublecortin expression levels in adult brain reflect neurogenesis. Eur J Neurosci 2005;21:1–14. https://doi.org/10.1111/j.1460-9568.2004.03813.x.

[9] Kandasamy M, Aigner L. Neuroplasticity, limbic neuroblastosis and neuro-regenerative disorders. Neural Regen Res 2018;13:1322–6. https://doi.org/10.4103/1673-5374.235214.

[10] Kempermann G, Song H, Gage FH. Neurogenesis in the adult hippocampus. Cold Spring Harb Perspect Biol 2015;7:a018812https://doi.org/10.1101/cshperspect.a018812.

[11] Kandasamy M, Couillard-Despres S, Raber KA, Stephan M, Lehner B, Winner B, et al. Stem cell quiescence in the hippocampal neurogenic niche is associated with elevated transforming growth factor-beta signaling in an animal model of Huntington disease. J Neuropathol Exp Neurol 2010;69:717–28. https://doi.org/10.1097/NEN.0b013e3181e4f733.

[12] Gonçalves JT, Schafer ST, Gage FH. Adult neurogenesis in the hippocampus: from stem cells to behavior. Cell 2016;167:897–914. https://doi.org/10.1016/j.cell.2016.10.021.

[13] Jhaveri DJ, Tedoldi A, Hunt S, Sullivan R, Watts NR, Power JM, et al. Evidence for newly generated interneurons in the basolateral amygdala of adult mice. Mol Psychiatry 2018;23:521–32. https://doi.org/10.1038/mp.2017.134.

[14] Paul A, Chaker Z, Doetsch F. Hypothalamic regulation of regionally distinct adult neural stem cells and neurogenesis. Science 2017;356:1383–6. https://doi.org/10.1126/science.aal3839.

[15] Kandasamy M, Aigner L. Reactive neuroblastosis in Huntington's disease: a putative therapeutic target for striatal regeneration in the adult brain. Front Cell Neurosci 2018;12:37. https://doi.org/10.3389/fncel.2018.00037.

[16] Ming G, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 2011;70:687–702. https://doi.org/10.1016/j.neuron.2011.05.001.

[17] Aimone JB, Li Y, Lee SW, Clemenson GD, Deng W, Gage FH. Regulation and function of adult neurogenesis: from genes to cognition. Physiol Rev 2014;94:991–1026. https://doi.org/10.1152/physrev.00004.2014.

[18] Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn AM, Nordborg C, Peterson DA, et al. Neurogenesis in the adult human hippocampus. Nat Med 1998;4:1313–7. https://doi.org/10.1038/3305.

[19] Gould E. How widespread is adult neurogenesis in mammals? Nat Rev Neurosci 2007;8:481–8. https://doi.org/10.1038/nrn2147.

[20] Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, et al. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature 2018;555:377–81. https://doi.org/10.1038/nature25975.

[21] Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, et al. Human hippocampal neurogenesis persists throughout aging. Cell Stem Cell 2018;22(589–599):e5https://doi.org/10.1016/j.stem.2018.03.015.

[22] Sanai N, Nguyen T, Ihrie RA, Mirzadeh Z, Tsai H-H, Wong M, et al. Corridors of migrating neurons in the human brain and their decline during infancy. Nature 2011;478:382–6. https://doi.org/10.1038/nature10487.

[23] Kempermann G, Gage FH, Aigner L, Song H, Curtis MA, Thuret S, et al. Human adult neurogenesis: evidence and remaining questions. Cell Stem Cell 2018;23:25–30. https://doi.org/10.1016/j.stem.2018.04.004.

[24] Kandasamy M, Rosskopf M, Wagner K, Klein B, Couillard-Despres S, Reitsamer HA, et al. Reduction in subventricular zone-derived olfactory bulb neurogenesis in a rat model of Huntington's disease is accompanied by striatal invasion of neuroblasts. PLoS ONE 2015;10:e0116069https://doi.org/10.1371/journal.pone.0116069.

[25] Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. Ann Neurol 2002;52:802–13. https://doi.org/10.1002/ana.10393.

[26] Sato H, Ishii Y, Yamamoto S, Azuma E, Takahashi Y, Hamashima T, et al. PDGFR-β plays a key role in the ectopic migration of neuroblasts in cerebral stroke. Stem Cells 2016;34:685–98. https://doi.org/10.1002/stem.2212.

[27] Tattersfield AS, Croon RJ, Liu YW, Kells AP, Faull RLM, Connor B. Neurogenesis in the striatum of the quinolinic acid lesion model of Huntington's disease. Neuroscience 2004;127:319–32. https://doi.org/10.1016/j.neuroscience.2004.04.061.

[28] Jessberger S, Parent JM. Epilepsy and adult neurogenesis. Cold Spring Harb Perspect Biol 2015;7.. https://doi.org/10.1101/cshperspect.a020677.

[29] Murphy BL, Pun RYK, Yin H, Faulkner CR, Loepke AW, Danzer SC. Heterogeneous integration of adult-generated granule cells into the epileptic brain. J Neurosci 2011;31:105–17. https://doi.org/10.1523/JNEUROSCI.2728-10.2011.

[30] Larson TA, Thatra NM, Lee BH, Brenowitz EA. Reactive neurogenesis in response to naturally occurring apoptosis in an adult brain. J Neurosci 2014;34:13066–76. https://doi.org/10.1523/JNEUROSCI.3316-13.2014.

[31] Periyasamy S, Sathya M, Karthick C, Kandasamy M, Shanmugaapriya S, Tamilselvan J, et al. Association studies of specific cholesterol related genes (APOE, LPL, and CETP) with lipid profile and memory function: a correlative study among rural and tribal population of Dharmapuri district, India. J Alzheimers Dis 2017;60:S195–207. https://doi.org/10.3233/JAD-170272.

[32] Maeder ML, Gersbach CA. Genome-editing technologies for gene and cell therapy. Mol Ther 2016;24:430–46. https://doi.org/10.1038/mt.2016.10.

[33] Gaj T, Sirk SJ, Shui S-L, Liu J. Genome-editing technologies: principles and applications. Cold Spring Harb Perspect Biol 2016:8. https://doi.org/10.1101/cshperspect.a023754.

[34] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–76. https://doi.org/10.1016/j.cell.2006.07.024.

[35] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72. https://doi.org/10.1016/j.cell.2007.11.019.

[36] Gurdon JB. Nuclear reprogramming and cell replacement therapies. Nat Rev Mol Cell Biol 2016;17:137–8.

[37] Grade S, Götz M. Neuronal replacement therapy: previous achievements and challenges ahead. NPJ Regen Med 2017;2:29. https://doi.org/10.1038/s41536-017-0033-0.

[38] Marchetto MCN, Winner B, Gage FH. Pluripotent stem cells in neurodegenerative and neurodevelopmental diseases. Hum Mol Genet 2010;19:R71–6. https://doi.org/10.1093/hmg/ddq159.

[39] Jung Y-W, Hysolli E, Kim K-Y, Tanaka Y, Park I-H. Human induced pluripotent stem cells and neurodegenerative disease: prospects for novel therapies. Curr Opin Neurol 2012;25:125–30. https://doi.org/10.1097/WCO.0b013e3283518226.

[40] Ross CA, Akimov SS. Human-induced pluripotent stem cells: potential for neurodegenerative diseases. Hum Mol Genet 2014;23:R17–26. https://doi.org/10.1093/hmg/ddu204.

[41] Pistollato F, Canovas-Jorda D, Zagoura D, Price A. Protocol for the differentiation of human induced pluripotent stem cells into mixed cultures of neurons and glia for neurotoxicity testing. J Vis Exp 2017. https://doi.org/10.3791/55702.

[42] Liang N, Trujillo CA, Negraes PD, Muotri AR, Lameu C, Ulrich H. Stem cell contributions to neurological disease modeling and personalized medicine. Prog Neuropsychopharmacol Biol Psychiatry 2018;80:54–62. https://doi.org/10.1016/j.pnpbp.2017.05.025.

[43] Medvedev SP, Shevchenko AI, Zakian SM. Induced pluripotent stem cells: problems and advantages when applying them in regenerative medicine. Acta Naturae 2010;2:18–28.

[44] Lee S-Y, Chung S-K. Integrating gene correction in the reprogramming and transdifferentiation processes: a one-step strategy to overcome stem cell-based gene therapy limitations. Stem Cells Int 2016:2016. https://doi.org/10.1155/2016/2725670.

[45] Frega M, van Gestel SHC, Linda K, van der Raadt J, Keller J, Van Rhijn J-R, et al. Rapid neuronal differentiation of induced pluripotent stem cells for measuring network activity on micro-electrode arrays. J Vis Exp 2017. https://doi.org/10.3791/54900.

[46] Ho S-M, Topol A, Brennand KJ. From "directed differentiation" to "neuronal induction": modeling neuropsychiatric disease. Biomark Insights 2015;10:31–41. https://doi.org/10.4137/BMI.S20066.

[47] Ohnuki M, Takahashi K. Present and future challenges of induced pluripotent stem cells. Philos Trans R Soc Lond B Biol Sci 2015:370. https://doi.org/10.1098/rstb.2014.0367.

[48] Paavilainen T, Pelkonen A, Mäkinen ME-L, Peltola M, Huhtala H, Fayuk D, et al. Effect of prolonged differentiation on functional maturation of human pluripotent stem cell-derived neuronal cultures. Stem Cell Res 2018;27:151–61. https://doi.org/10.1016/j.scr.2018.01.018.

[49] Liang G, Zhang Y. Genetic and epigenetic variations in iPSCs: potential causes and implications for application. Cell Stem Cell 2013;13:149–59. https://doi.org/10.1016/j.stem.2013.07.001.

[50] Kyttälä A, Moraghebi R, Valensisi C, Kettunen J, Andrus C, Pasumarthy KK, et al. Genetic variability overrides the impact of parental cell type and determines iPSC differentiation potential. Stem Cell Rep 2016;6:200–12. https://doi.org/10.1016/j.stemcr.2015.12.009.

[51] Seki T. Expression patterns of immature neuronal markers PSA-NCAM, CRMP-4 and NeuroD in the hippocampus of young adult and aged rodents. J Neurosci Res 2002;70:327–34. https://doi.org/10.1002/jnr.10387.

[52] Brown JP, Couillard-Després S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG. Transient expression of doublecortin during adult neurogenesis. J Comp Neurol 2003;467:1–10. https://doi.org/10.1002/cne.10874.

[53] Zhang J, Jiao J. Molecular biomarkers for embryonic and adult neural stem cell and neurogenesis. Biomed Res Int 2015;2015. https://doi.org/10.1155/2015/727542.
[54] Gao Z, Ure K, Ables JL, Lagace DC, Nave K-A, Goebbels S, et al. Neurod1 is essential for the survival and maturation of adult-born neurons. Nat Neurosci 2009;12:1090–2. https://doi.org/10.1038/nn.2385.
[55] Kim EJ, Ables JL, Dickel LK, Eisch AJ, Johnson JE. Ascl1 (Mash1) defines cells with long-term neurogenic potential in subgranular and subventricular zones in adult mouse brain. PLoS One 2011;6. https://doi.org/10.1371/journal.pone.0018472.
[56] Cremer H, Chazal G, Lledo PM, Rougon G, Montaron MF, Mayo W, et al. PSA-NCAM: an important regulator of hippocampal plasticity. Int J Dev Neurosci 2000;18:213–20.
[57] Lavado A, Lagutin OV, Chow LML, Baker SJ, Oliver G. Prox1 is required for granule cell maturation and intermediate progenitor maintenance during brain neurogenesis. PLoS Biol 2010;8. https://doi.org/10.1371/journal.pbio.1000460.
[58] Brandt MD, Jessberger S, Steiner B, Kronenberg G, Reuter K, Bick-Sander A, et al. Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. Mol Cell Neurosci 2003;24:603–13.
[59] Ayanlaja AA, Xiong Y, Gao Y, Ji G, Tang C, Abdikani Abdullah Z, et al. Distinct features of doublecortin as a marker of neuronal migration and its implications in cancer cell mobility. Front Mol Neurosci 2017:10. https://doi.org/10.3389/fnmol.2017.00199.
[60] Rotheneichner P, Belles M, Benedetti B, König R, Dannehl D, Kreutzer C, et al. Cellular plasticity in the adult murine piriform cortex: continuous maturation of dormant precursors into excitatory neurons. Cereb Cortex 2018;28:2610–21. https://doi.org/10.1093/cercor/bhy087.
[61] Gleeson JG, Allen KM, Fox JW, Lamperti ED, Berkovic S, Scheffer I, et al. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell 1998;92:63–72.
[62] Gleeson JG, Lin PT, Flanagan LA, Walsh CA. Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. Neuron 1999;23:257–71.
[63] Horesh D, Sapir T, Francis F, Wolf SG, Caspi M, Elbaum M, et al. Doublecortin, a stabilizer of microtubules. Hum Mol Genet 1999;8:1599–610.
[64] Koizumi H, Higginbotham H, Poon T, Tanaka T, Brinkman BC, Gleeson JG. Doublecortin maintains bipolar shape and nuclear translocation during migration in the adult forebrain. Nat Neurosci 2006;9:779–86. https://doi.org/10.1038/nn1704.
[65] Filipovic R, Kumar SS, Fiondella C, Loturco J. Increasing doublecortin expression promotes migration of human embryonic stem cell-derived neurons. Stem Cells 2012;30:1852–62. https://doi.org/10.1002/stem.1162.
[66] Rao MS, Shetty AK. Efficacy of doublecortin as a marker to analyse the absolute number and dendritic growth of newly generated neurons in the adult dentate gyrus. Eur J Neurosci 2004;19:234–46.
[67] Feliciano DM, Bordey A, Bonfanti L. Noncanonical sites of adult neurogenesis in the mammalian brain. Cold Spring Harb Perspect Biol 2015;7:a018846https://doi.org/10.1101/cshperspect.a018846.
[68] Couillard-Despres S, Wuertinger C, Kandasamy M, Caioni M, Stadler K, Aigner R, et al. Ageing abolishes the effects of fluoxetine on neurogenesis. Mol Psychiatry 2009;14:856–64. https://doi.org/10.1038/mp.2008.147.
[69] Kempermann G, Gast D, Gage FH. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann Neurol 2002;52:135–43. https://doi.org/10.1002/ana.10262.
[70] Klempin F, Kronenberg G, Cheung G, Kettenmann H, Kempermann G. Properties of doublecortin-(DCX)-expressing cells in the piriform cortex compared to the neurogenic dentate gyrus of adult mice. PLoS ONE 2011;6:e25760https://doi.org/10.1371/journal.pone.0025760.
[71] Spampanato J, Sullivan RK, Turpin FR, Bartlett PF, Sah P. Properties of doublecortin expressing neurons in the adult mouse dentate gyrus. PLoS ONE 2012:7. https://doi.org/10.1371/journal.pone.0041029.
[72] Oboti L, Peretto P. How neurogenesis finds its place in a hardwired sensory system. Front Neurosci 2014;8.. https://doi.org/10.3389/fnins.2014.00102.
[73] Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature 1997;386:493–5. https://doi.org/10.1038/386493a0.
[74] van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 1999;2:266–70. https://doi.org/10.1038/6368.
[75] Larson TA. Sex steroids, adult neurogenesis, and inflammation in CNS homeostasis, degeneration, and repair. Front Endocrinol (Lausanne) 2018;9. https://doi.org/10.3389/fendo.2018.00205.
[76] Kempermann G. Activity dependency and aging in the regulation of adult neurogenesis. Cold Spring Harb Perspect Biol 2015:7. https://doi.org/10.1101/cshperspect.a018929.
[77] Torner L, Karg S, Blume A, Kandasamy M, Kuhn H-G, Winkler J, et al. Prolactin prevents chronic stress-induced decrease of adult hippocampal neurogenesis and promotes neuronal fate. J Neurosci 2009;29:1826–33. https://doi.org/10.1523/JNEUROSCI.3178-08.2009.
[78] Li C, Zhang Y-X, Yang C, Hao F, Chen S-S, Hao Q, et al. Intraventricular administration of endoneuraminidase-N facilitates ectopic migration of subventricular zone-derived neural progenitor cells into 6-OHDA lesioned striatum of mice. Exp Neurol 2016;277:139–49. https://doi.org/10.1016/j.expneurol.2015.12.017.
[79] Cayre M, Canoll P, Goldman JE. Cell migration in the normal and pathological postnatal mammalian brain. Prog Neurobiol 2009;88:41–63. https://doi.org/10.1016/j.pneurobio.2009.02.001.
[80] Saha B, Jaber M, Gaillard A. Potentials of endogenous neural stem cells in cortical repair. Front Cell Neurosci 2012;6. https://doi.org/10.3389/fncel.2012.00014.
[81] Yamashita T, Ninomiya M, Acosta PH, García-Verdugo JM, Sunabori T, Sakaguchi M, et al. Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. J Neurosci 2006;26:6627–36. https://doi.org/10.1523/JNEUROSCI.0149-06.2006.
[82] Unger MS, Marschallinger J, Kaindl J, Höfling C, Rossner S, Heneka MT, et al. Early changes in hippocampal neurogenesis in transgenic mouse models for Alzheimer's disease. Mol Neurobiol 2016;53:5796–806. https://doi.org/10.1007/s12035-016-0018-9.
[83] Han M-H, Lee E-H, Koh S-H. current opinion on the role of neurogenesis in the therapeutic strategies for Alzheimer disease, Parkinson disease, and ischemic stroke; considering neuronal voiding function. Int Neurourol J 2016;20:276–87. https://doi.org/10.5213/inj.1632776.388.
[84] Galán L, Gómez-Pinedo U, Guerrero A, García-Verdugo JM, Matías-Guiu J. Amyotrophic lateral sclerosis modifies progenitor neural proliferation in adult classic neurogenic brain niches. BMC Neurol 2017;17:173. https://doi.org/10.1186/s12883-017-0956-5.
[85] Unger MS, Marschallinger J, Kaindl J, Klein B, Johnson M, Khundakar AA, et al. Doublecortin expression in CD8+ T-cells and microglia at sites of amyloid-β plaques: a potential role in shaping plaque pathology? Alzheimers Dement 2018;14:1022–37. https://doi.org/10.1016/j.jalz.2018.02.017.
[86] Rakic P. Adult neurogenesis in mammals: an identity crisis. J Neurosci 2002;22:614–8.
[87] Baptista P, Andrade JP. Adult hippocampal neurogenesis: regulation and possible functional and clinical correlates. Front Neuroanat 2018:12. https://doi.org/10.3389/fnana.2018.00044.
[88] Couillard-Despres S, Finkl R, Winner B, Ploetz S, Wiedermann D, Aigner R, et al. In vivo optical imaging of neurogenesis: watching new neurons in the intact brain. Mol Imaging 2008;7:28–34.
[89] Fricke IB, Schelhaas S, Zinnhardt B, Viel T, Hermann S, Couillard-Després S, et al. In vivo bioluminescence imaging of neurogenesis – the role of the blood brain barrier in an experimental model of Parkinson's disease. Eur J Neurosci 2017;45:975–86. https://doi.org/10.1111/ejn.13540.
[90] Curtis MA, Penney EB, Pearson AG, van Roon-Mom WMC, Butterworth NJ, Dragunow M, et al. Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. Proc Natl Acad Sci USA 2003;100:9023–7. https://doi.org/10.1073/pnas.1532244100.
[91] Winner B, Winkler J. Adult neurogenesis in neurodegenerative diseases. Cold Spring Harb Perspect Biol 2015:7. https://doi.org/10.1101/cshperspect.a021287.
[92] Hodes RJ. Telomere length, aging, and somatic cell turnover. J Exp Med 1999;190:153–6.
[93] Geraghty RJ, Capes-Davis A, Davis JM, Downward J, Freshney RI, Knezevic I, et al. Guidelines for the use of cell lines in biomedical research. Br J Cancer 2014;111:1021–46. https://doi.org/10.1038/bjc.2014.166.
[94] Dolmetsch R, Geschwind DH. The human brain in a dish: the promise of iPSC-derived neurons. Cell 2011;145:831–4. https://doi.org/10.1016/j.cell.2011.05.034.
[95] Park I-H, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, et al. Disease-specific induced pluripotent stem cells. Cell 2008;134:877–86. https://doi.org/10.1016/j.cell.2008.07.041.
[96] Fuller HR, Mandefro B, Shirran SL, Gross AR, Kaus AS, Botting CH, et al. Spinal muscular atrophy patient iPSC-derived motor neurons have reduced expression of proteins important in neuronal development. Front Cell Neurosci 2016;9.. https://doi.org/10.3389/fncel.2015.00506.
[97] Yagi T, Ito D, Okada Y, Akamatsu W, Nihei Y, Yoshizaki T, et al. Modeling familial Alzheimer's disease with induced pluripotent stem cells. Hum Mol Genet 2011;20:4530–9. https://doi.org/10.1093/hmg/ddr394.
[98] Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 2009;136:964–77. https://doi.org/10.1016/j.cell.2009.02.013.
[99] Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 2008;321:1218–21. https://doi.org/10.1126/science.1158799.
[100] Deshpande A, Yadav S, Dao DQ, Wu Z-Y, Hokanson KC, Cahill MK, et al. Cellular phenotypes in human iPSC-derived neurons from a genetic model of autism spectrum disorder. Cell Rep 2017;21:2678–87. https://doi.org/10.1016/j.celrep.2017.11.037.
[101] Hoffman GE, Hartley BJ, Flaherty E, Ladran I, Gochman P, Ruderfer DM, et al. Transcriptional signatures of schizophrenia in hiPSC-derived NPCs and neurons are concordant with post-mortem adult brains. Nat Commun 2017;8:2225. https://doi.org/10.1038/s41467-017-02330-5.
[102] Hu B-Y, Weick JP, Yu J, Ma L-X, Zhang X-Q, Thomson JA, et al. Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. Proc Natl Acad Sci USA 2010;107:4335–40. https://doi.org/10.1073/pnas.0910012107.
[103] Kim D-S, Ross PJ, Zaslavsky K, Ellis J. Optimizing neuronal differentiation from induced pluripotent stem cells to model ASD. Front Cell Neurosci 2014:8. https://doi.org/10.3389/fncel.2014.00109.
[104] Prasad A, Manivannan J, Loong DT, Chua SM, Gharibani PM, All AH. A review of induced pluripotent stem cell, direct conversion by trans-differentiation, direct reprogramming and oligodendrocyte differentiation. Regenerative Med 2016;11:181–91. https://doi.org/10.2217/rme.16.5.
[105] Bamba Y, Shofuda T, Kato M, Pooh RK, Tateishi Y, Takanashi J-I, et al. In vitro characterization of neurite extension using induced pluripotent stem cells derived

from lissencephaly patients with TUBA1A missense mutations. Mol Brain 2016;9:70. https://doi.org/10.1186/s13041-016-0246-y.
[106] Pilz DT, Matsumoto N, Minnerath S, Mills P, Gleeson JG, Allen KM, et al. LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. Hum Mol Genet 1998;7:2029–37.
[107] Germain J, Bruel-Jungerman E, Grannec G, Denis C, Lepousez G, Giros B, et al. Doublecortin knockout mice show normal hippocampal-dependent memory despite CA3 lamination defects. PLoS ONE 2013;8:e74992https://doi.org/10.1371/journal.pone.0074992.
[108] Dhaliwal J, Xi Y, Bruel-Jungerman E, Germain J, Francis F, Lagace DC. Doublecortin (DCX) is not essential for survival and differentiation of newborn neurons in the adult mouse dentate gyrus. Front Neurosci 2016:9. https://doi.org/10.3389/fnins.2015.00494.
[109] Malik N, Rao MS. A review of the methods for human iPSC derivation. Methods Mol Biol 2013;997:23–33. https://doi.org/10.1007/978-1-62703-348-0_3.
[110] Rao MS, Malik N. Assessing iPSC reprogramming methods for their suitability in translational medicine. J Cell Biochem 2012;113:3061–8. https://doi.org/10.1002/jcb.24183.