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Cell-reprogramming technology and neuroscience

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Reprogramming technology enables differentiated cells of a specific cell type to be converted to another cell type with completely different functions, either through the production of induced pluripotent stem cells (iPSCs) or through direct conversion. This technology has challenged the idea that differentiated cells types are immutable entities and has enabled researchers to study the behaviour of living cell types that were previously inaccessible, such as human neurons. The study of live human neurons in a dish allows human-specific neurodevelopmental properties to be identified and the specific pathways that are defective in cells from patients with neuropsychiatric and neurodegenerative diseases to be dissected. Integrating methods to differentiate iPSCs into the relevant cell types involved in neurological disease with reproducible and scalable phenotypic assays is a new challenge for the disease-modelling field.

Applications of iPSC-derived neural cells

Understanding basic principles of human brain development

iPSC-derived neurons can be used to determine the neurodevelopmental hallmarks of human neural cells (neurons and glia) and to investigate the epigenetic landscape of the cells during differentiation and the relationship between epigenetics and gene regulation. For example, given that epigenetic changes can influence gene expression and cell fate determination over time, it is highly informative to study the influence of epigenetics during human neural differentiation — something that is only possible now owing to the use of iPSC technology. These cells can also be used to study the characteristics of human iPSC-derived neural cells versus those derived from other non-human primates that are our closest relatives (such as chimpanzees). Such experiments can provide clues as to how humans evolved such a unique brain.

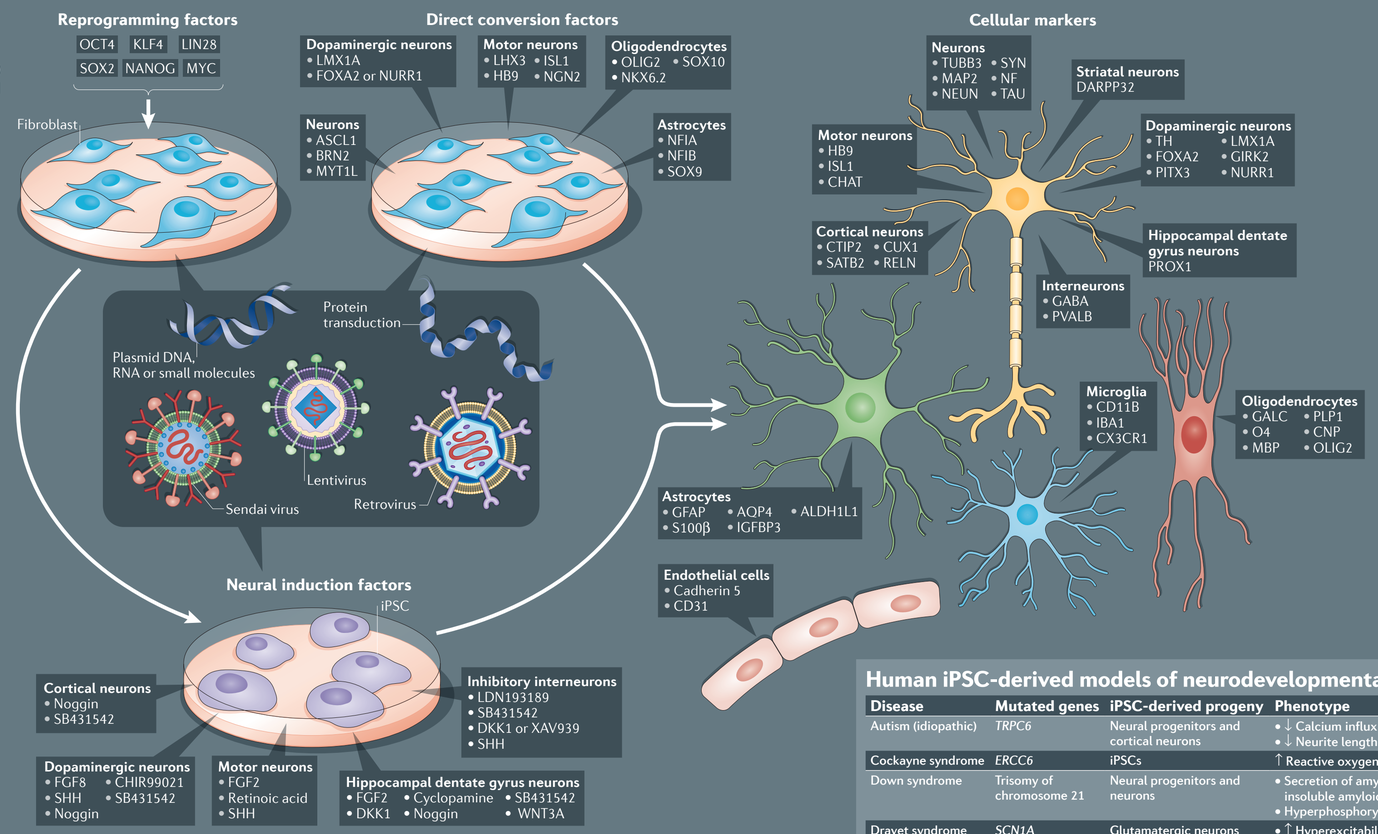
Understanding and treating CNS disease

Cell-reprogramming technology (see central figure) has remarkable potential to generate insights into disease mechanisms, particularly in the case of CNS diseases. Researchers can use reprogramming technology to study human disease in living neural cells that carry disease-specific genetic variants (see Tables). By comparing cells derived from patients and controls or manipulating gene expression in different neuronal subtypes using gene editing, researchers can gain an understanding of basic disease mechanisms.

Studying the development of neural cells derived from patient iPSCs will facilitate our understanding of the early steps of CNS disease processes and could therefore provide new early diagnostic tools and disease biomarkers. iPSC-derived cells can also be used in high-throughput assays for drug screening (see right figure). Indeed, reprogramming technology is already informing clinical trials. For example, iPSC-derived human neurons from patients with autism spectrum disorders that were treated with IGF1 exhibited a significantly improved phenotype *in vitro*. Modified versions of IGF1 are now in clinical trials for patients with several types of autistic spectrum disorder. It remains a challenge to predict the molecules that will work both *in vitro* and *in vivo*, but the prospect that these new models can help us to understand and potentially treat CNS diseases is exciting.

iPSC technology may also allow for the development of patient-tailored therapies: drug screening can be performed on the cells from the patient that will potentially receive the therapy, decreasing the effect of genetic background variability among individuals.

Cellular replacement therapy is also an exciting application of iPSC technology. The first patients are already receiving iPSC-derivatives via transplantation for some neurological diseases; however, caution must be taken owing to the tumorigenic potential of pluripotent stem cells.



High-throughput phenotypic assays using iPSC-derived cells

iPSC-derived cells from patients and control populations

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High-throughput phenotypic screen:
- RNA sequencing and single-cell sequencing technology
- Flow cytometry
- Measurements of cell morphology
- Immunohistochemistry
- Calcium imaging
- Multi-electrode arrays
- Microfluidics

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Automated platform for data analysis and integration of data from different assays

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Identification of quantifiable differences in measurements between patients and controls

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Screening of drugs

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Clinical trials

Human iPSC-derived models of neurodegenerative disorders

Disease	Mutated genes	iPSC-derived progeny	Phenotype
Adrenoleukodystrophy	*ABCD1*	Oligodendrocytes and neurons	↑ Levels of very-long-chain fatty acids
Alzheimer disease	• *PSEN1* • *PSEN2* • *APP*	Cortical neurons	• ↑ Amyloid-β secretion • ↑ Phospho-tau (Thr 231) • ↑ Active GSK3β
Amyotrophic lateral sclerosis	• *SOD1* • *VAPB* • *TARDBP*	Motor neurons and glial cells	• ↓ VAPB • ↑ TDP43
Familial dysautonomia	*IKBKAP*	Neural crest progenitor cells	• ↓ Neurogenesis and differentiation genes • Defects in neural crest migration
Friedreich ataxia	*FXN*	Neurons	↓ Frataxin protein levels
Hereditary spastic paraplegia	*SPG14*	Corticospinal motor neurons	• ↓ Neurite complexity • ↑ Neurite swellings • Impaired axonal transport
Huntington disease	*HTT*	Neural stem cells and astrocytes	• Susceptibility to stress • Vulnerability to BDNF withdrawal • ↑ Cell death • ↑ Protein aggregate inclusions • Altered mitochondria bioenergetics
Machado–Joseph disease	*ATXN3*	Glutamatergic neurons	Excitation-induced ataxin 3 aggregation
Parkinson disease	• *LRRK2* • *PINK1* • *SNCA* • *PARK2* • *GBA3*	Dopaminergic neurons	• Impaired mitochondrial function • Sensitivity to oxidative stress • ↓ Dopamine reuptake • ↑ Spontaneous dopamine release • ↑ α-synuclein
Spinal and bulbar muscular atrophy	CAG repeat in the androgen receptor gene	Motor neurons	• ↑ Aggregation of androgen receptor protein inclusions • Autophagy defects
Spinal muscular atrophy	*SMN1*	Motor neurons	↓ Size and number

Challenges and future directions

Modelling polygenic and multifactorial CNS disorders using cells derived through reprogramming requires an integrative approach that combines the capacity to detect relevant genetic determinants with the ability to search for related pathways and cellular phenotypes in reproducible and scalable bioassays. There remain several technical challenges to be overcome:

- Generating mature, adult-like neural cell types and refining the methods for detecting the maturation of neuronal activity
- Identifying genomic mutations that result in robust phenotypes in iPSC-derived cells
- Improving reproducibility and scalability of phenotypic assays
- Incorporating gene-editing technologies (such as the CRISPR–Cas9 system) to correct relevant changes in iPSCs to better understand disease mechanisms and potentially create cells for transplantation strategies
- Incorporating other CNS niche cells (for example, astrocytes, oligodendrocytes and microglia) and/or inflammatory factors into the *in vitro* model to produce more-disease-relevant phenotypes
- Incorporating the use of 3D culture models ('organoid' technology) in order to create more-realistic models of development and disease (for example, by simulating cortical layer formation)
- Developing screening platforms for new compounds that can be readily translated to *in vivo* experiments

Human iPSC-derived models of neurodevelopmental and psychiatric disorders

Disease	Mutated genes	iPSC-derived progeny	Phenotype
Autism (idiopathic)	*TRPC6*	Neural progenitors and cortical neurons	• ↓ Calcium influx • ↓ Neurite length, complexity and spine density
Cockayne syndrome	*ERCC6*	iPSCs	↑ Reactive oxygen species
Down syndrome	Trisomy of chromosome 21	Neural progenitors and neurons	• Secretion of amyloid-β isoform 42 and formation of insoluble amyloid aggregates • Hyperphosphorylated tau on cell bodies and dendrites
Dravet syndrome	*SCN1A*	Glutamatergic neurons	• ↑ Hyperexcitability • ↑ Persistent sodium channel activation • ↑ Evoked action potentials
Fragile X syndrome	*FMR1*	Forebrain neurons	Defective neurite initiation and extension
Phelan–McDermid syndrome	• 22q13.3 deletion • *SHANK3*	Forebrain neurons	• Defects in excitatory synaptic transmission • ↓ Glutamate receptor expression and number of synapses
Rett syndrome	• *MECP2* • *CDKL5*	Neurons and neural progenitors	• Neuronal maturation defects • ↓ Synapse and spine number • ↓ Soma size • ↑ LINE1 retrotransposition • Aberrant dendritic spines
Schizophrenia	*DISC1*	Neurons and hippocampal dentate gyrus neurons	• ↓ Neuronal connectivity and neurite number • ↓ PSD95 and glutamate receptor expression • ↑ Extra-mitochondrial oxygen consumption • ↑ Reactive oxygen species • ↓ Spontaneous neurotransmitter release in dentate gyrus neurons
Timothy syndrome	*CACNA1C*	Cortical neurons	• ↓ Expression of lower cortical layer and callosal projection genes • Abnormal expression of TH • ↑ Production of noradrenaline and dopamine • Activity-dependent dendritic retraction

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