Researchers develop new method for specializing and purifying human stem cells into interneurons

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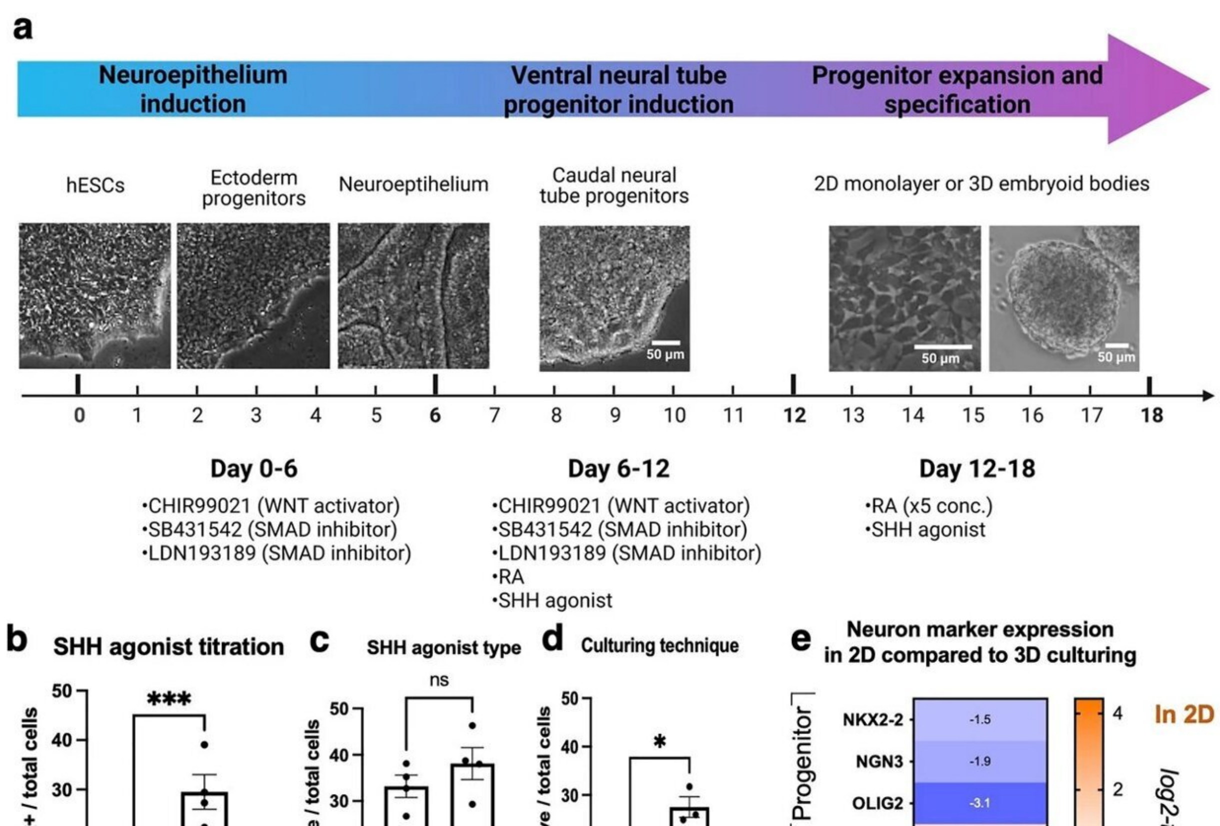

Optimization of hESC differentiation towards NKX2-2$^+$ V3 progenitors. Optimization steps were analyzed on d18, unless specified otherwise. (**a**) A timeline of differentiation up to day 18, with representative bright field images for each stage. Differentiation was performed in basal media (Supplementary Table S2) with molecules indicated in the diagram. Schematic has been created using a template from Biorender.com. (**b**) SHH agonist purmorphamine titration

between d6–d18; 3D culture between d12-d18. Shown as mean ± s.e.m. (standard error of mean); n = 4. Two-tailed paired t-test, *p = 0.0003. (**c**) Comparison of the two SHH agonists between d6-d18; 3D culture between d12–d18. Performed in one differentiation, separate plates expressed as replicates. Shown as mean ± s.e.m; n = 4. Two-tailed paired t-test, p = 0.2983, n.s. SAG—smoothened agonist. (**d**) *2D*: d12 progenitors dissociated into a single cell 2D monolayer; *3D*: cells dissociated into clumps and plated as embryoid bodies in suspension; cultured in 0.5 μM purmorphamine. Shown as mean ± s.e.m.; n = 3. Two-tailed paired t-test, *p = 0.0159. (**e**) Relative gene expression by qRT-PCR, normalized to the housekeeping gene TATA box binding protein (TBP). Shown as a log2-transformed fold change of 2D- to 3D-cultured cells. Analyzed on d25. (**f**) Images show V3 progenitors ($NKX2\text{-}2^{+}$) and total cells (DAPI) derived using protocols before and after final optimization. (**g**) Neural progenitors derived with the optimized protocol matured on a mESC-astrocytic monolayer expressing glial cell line-derived neurotrophic factor (GDNF) until d67. Presynaptic marker example—Bassoon, *NFAA* neurofilament associated antigen, *VGLUT2* vesicular glutamate transporter 2—glutamatergic interneuron marker. Credit: *Scientific Reports* (2023). DOI: 10.1038/s41598-023-29165-z

Injury to the spinal cord often leads life changing disability, with decreased or complete loss of sensation and movement below the site of injury. From drugs to transplantation, there are many scientific advances aiming to restore function following spinal cord injury.

One promising approach is the use of stem cell derived neurons to replace those damaged. New research from the Centre for Gene Therapy & Regenerative Medicine and Centre for Neurodevelopment at King's College London hopes to improve on this approach by providing pure populations of neurons made from stem cells.

The spinal cord is a delicate structure, with neurons carry messages from your brain to the rest of your body to allow movement and sensation. Integral to this system are interneurons, or the cells that relay

information between your brain and other neurons. Research has previously shown that transplanting a class of interneurons, ventral spinal interneurons, to treat spinal cord injury in animal models provides promising recovery of sensory and motor function.

However, the use of these interneurons in human transplantation or to study on a larger scale is made difficult by their limited number after isolation from embryonic spinal cord tissue. In addition, the many different types of interneurons fall into two main classes: inhibitory and excitatory. Excitatory interneurons are more promising for cell therapy, as they relay information, rather than suppressing it. To overcome this issue, large quantities of excitatory interneurons may be made from human stem cells.

The new paper in *Scientific Reports* outlines an effective method for growing and purifying populations of an early form of excitatory ventral spinal interneurons, known as progenitors. The method builds on decades of understanding of neuron development in the embryo, applying chemical factors that mimic this process to direct specialization of embryonic stem cells to a mixed population of neuron cells.

Amongst them, interneuron progenitors can be identified thanks to genetic alteration which labels a receptor on the cell surface. The cells with this label can then be isolated to achieve an incredible 95% purity. Following isolation, the cells are further matured in fully functioning ventral spinal interneurons.

"[These results] are of great significance for the field of spinal cord research. Ventral spinal interneurons are an integral part of local spinal neural networks and a strategy to derive this cell type from human stem cells will undoubtedly have an enormous impact on developmental studies, disease modeling and cell therapies in spinal cord research," says Federica Riccio, Ph.D. student and second author.

Medical Xpress

More information: Ieva Berzanskyte et al, Enrichment of human embryonic stem cell-derived V3 interneurons using an Nkx2-2 gene-specific reporter, *Scientific Reports* (2023). DOI: 10.1038/s41598-023-29165-z

Provided by King's College London

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