STEM CELLS

Fast track to neurons

New protocols generate neurons directly from fibroblasts using only chemical cocktails.

Today's alchemy offers many ways to make a neuron. Pluripotent or neural stem cells can be coaxed toward mature neural fates, and terminally differentiated somatic cells can be converted directly into neurons. Protocols abound. But that hasn't stopped the drive to find methods that are more efficient, more expedient and potentially safer for human therapy.

Two Chinese teams—one led by Zhen Chai, Yang Zhao and Hongkui Deng of Peking University, and the other led by Jian Zhao and Gang Pei of the Shanghai Institute of Biological Sciences, Chinese Academy of Sciences and Tonji University—have been working toward these goals.

Jian Zhao says that *in vitro* differentiation of stem cells into neurons is still time-consuming and complicated. Direct conversion of somatic cells can offer a faster route but relies on the expression of lineage-specific transcription factors, typically from viral vectors. Recent work showed that cell-permeable small molecules can boost conversion efficiency, which spurred both teams to develop methods that dispense with ectopic gene expression altogether.

Small molecules have inherent advantages that include a lower risk of generating unwanted mutations (there is no genetic integration), low immunogenicity, ease of use and excellent dosage control. However, Yang Zhao and Deng point out that fine-tuning the concentration and duration of drug application is critical. Which cellular pathways the molecules target, and their specificity, is also often unknown.

Yang Zhao, Deng, Chai and their colleagues carried out two rounds of screening for chemicals that convert mouse skin cells into neurons, first in the presence of the neuronal regulator Ascl1, and then without it. Their final cocktail caused up to 90% of cells to express the TUJ1 neuronal marker within 16 days, and further maturation imbued over half of 39 tested cells with functional membrane properties and the ability to fire action potentials. Coculture experiments with astrocytes or primary neurons demonstrated that the cells were capable of forming functional synaptic connections with each other and with existing neurons.

The researchers found that the ISX9 molecule was essential for activating neuron-specific genes, and a BET-family bromodomain inhibitor that can affect chromatin remodeling caused a loss of the skin-cell program, its first known role in reprogramming. Yang Zhao and Deng believe that epigenetic factors may act by erasing initial cell identities, enabling the acquisition of converted fates.

Jian Zhao and Pei's team focused on human cells, starting with a cocktail they previously developed to generate neuronal progenitors from somatic cells and then adding small molecules that promote neuron survival and maturation. Their seven-molecule mixture converted about 5% of human fibroblasts into TUJ1-expressing neurons with morphology, gene expression profiles and electrophysiological properties similar to those of pluripotent stem cell–derived neurons and transcription factor–induced neurons. The researchers were also able to convert fibroblasts from two patients with Alzheimer's disease at similar efficiency, generating personalized cellular disease models.

The mouse and human protocols generate a mixture of excitatory and inhibitory cell types, with glutamatergic cells making up the majority, and both teams cite the need for approaches that generate specific neuronal types at higher purities. Jian Zhao and Pei's groups are studying the mechanism of their cocktail, and the Peking University scientists are keen to modify their protocol to generate more mature neurons, especially from human cells.

Direct chemical conversion may offer an alternative path for generating cell types other than neurons, providing disease models and possibilities for regenerative therapy.

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RESEARCH PAPERS

Li, X. *et al.* Small-molecule-driven direct reprogramming of mouse fibroblasts into functional neurons. *Cell Stem Cell* **17**, 195–203 (2015).

Hu, W. *et al.* Direct conversion of normal and Alzheimer's disease human fibroblasts into neuronal cells by small molecules. *Cell Stem Cell* **17**, 204–212 (2015).