THE AUTHOR FILE

Oliver Brüstle

Making direct conversion pure and efficient

Graduate students and postdoctoral fellows stay with principal investigators only for a short while. Watching them move on and establish themselves brings senior scientists a sense of satisfaction, says Oliver Brüstle, a stem-cell biologist at the University of Bonn. "You get the feeling that you contributed a little bit."

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But when Brüstle's former postdoc Marius Wernig published papers that described a way to convert mouse and human fibroblasts into neurons, Brüstle felt not only pleased but inspired. Previously reported techniques to make patient-specific neurons were laborious. Fibroblasts first had to be converted into induced pluripotent stem (iPS) cell lines. These had to be validated and characterized before being differentiated into neurons. Skipping the pluripotent stage could save weeks of time and effort.

But showing that neurons can be made directly from fibroblasts is a long way from making enough neurons for new experiments. In Wernig's proof-of-principle demonstrations, only a small fraction of fibroblasts became neurons, and because neurons are nondividing cells, the quantities produced were limited. Brüstle was keen to tweak Wernig's original conversion recipe—adding four transcription factors to cultured fibroblasts—to make it easier and more efficient. Brüstle's colleague and co-corresponding author Philipp Koch felt exactly the same way. "He read the publications from Marius; the very same day, he started to clone the vectors that carried genes for the transcription factors," says Brüstle.

Postdoc Julia Ladewig took charge of systematically testing many theoretically promising combinations of transcription factors and small molecules that might affect relevant pathways, eventually formulating a recipe with two small molecules and two transcription factors. "I remember very well when I saw the first data," says Brüstle. The flat fibroblasts were overwhelmed by "these beautiful neurons with processes all over the dish." He recalls his surprise. "I thought, 'That can't be; that is such a tremendous number of neurons.'" His team then checked the starting fibroblasts for abnormalities and confirmed their results with other batches of cells.

After that excitement came weeks of tedium. To show definitively that the neurons were functional, Brüstle's team had to complete a grueling series of electrophysiology experiments, all the while worrying that other researchers would beat them to publication of a high-yielding recipe. Ultimately, they were able to show that their protocol, which used human postnatal fibroblasts from donors ranging from newborn to four years old, produced populations of neurons with yields over 200% (fibroblasts continue to divide during conversion) and purity as high as 80%.

But success with this protocol is bittersweet. The small molecules in the conversion recipe were known from work differentiating human embryonic stem (ES) cells, a class of research recently condemned by the European Court of Justice. In 2004, the environmental group Greenpeace challenged Brüstle's patent to differentiate human ES cells into neurons. When the lawsuit ended up before a pan-European body, Brüstle was initially elated. German laws are the most restrictive on the continent, he explains, so he thought any changes would only liberalize policy. Instead, the court banned patents involving procedures on human ES cells throughtout the European Union.

Brüstle believes the decision weakens not only embryonic stem cell science, but also research in reprogramming and direct conversion. "The field is cross-fertilized by developments pushed forward using embryonic stem cells," he says. "Looking in the toolbox we have now, one tool is ES cells, another is iPS cells, and the emerging technology is direct conversion. Today, the idea that any of these will somehow outperform the others and make [it] indispensible is naive."

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Right now, though, Brüstle is focusing on making direct conversion the best tool it can be. As the field develops, researchers will need a framework to evaluate fate shifts, and Brüstle believes two parameters will be key. One is 'yield', which he describes as the number of neurons obtained relative to the starting population of fibroblasts. The second parameter is 'purity', the percentage of neurons in the final preparation.

Assessment of how well fibroblast-induced neurons resemble their *in vivo* counterparts is also crucial, says Brüstle, and so is additional work to efficiently produce neurons from fibroblasts taken from patients with relevant diseases, which can then be used to model disease and screen compounds. Such advances are coming, Brüstle believes. "We hope this will enable efficient production of neurons from large cohorts of patients within a very short time."

Monya Baker

Ladewig, J. *et al.* Small molecules enable highly efficient neuronal conversion of human fibroblasts. *Nat. Methods* **9**, 575-578 (2012).