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Conversion of Human Fibroblasts to Stably Self-Renewing Neural Stem Cells with a Single Zinc-Finger Transcription Factor

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SUMMARY

Direct conversion of somatic cells into neural stem cells (NSCs) by defined factors holds great promise for mechanistic studies, drug screening, and potential cell therapies for different neurodegenerative diseases. Here, we report that a single zinc-finger transcription factor, *Zfp521*, is sufficient for direct conversion of human fibroblasts into long-term self-renewable and multipotent NSCs. In vitro, *Zfp521*-induced NSCs maintained their characteristics in the absence of exogenous factor expression and exhibited morphological, molecular, developmental, and functional properties that were similar to control NSCs. In addition, the single-seeded induced NSCs were able to form NSC colonies with efficiency comparable with control NSCs and expressed NSC markers. The converted cells were capable of surviving, migrating, and attaining neural phenotypes after transplantation into neonatal mouse and adult rat brains, without forming tumors. Moreover, the *Zfp521*-induced NSCs predominantly expressed rostral genes. Our results suggest a facilitated approach for establishing human NSCs through *Zfp521*-driven conversion of fibroblasts.

INTRODUCTION

Differentiated cells can be reprogrammed to become induced pluripotent stem cells (iPSCs) by exogenous supplementation of defined factors (Takahashi and Yamanaka, 2006). The iPSCs provide an interminable source of a broad range of differentiated cells for applications such as in vitro disease modeling, drug development, toxicity testing, and cell-replacement therapies. Mature neurons and neural stem cells (NSCs) are among the most clinically useful cells that can be produced from pluripotent stem cells (PSCs) (Nemati et al., 2011). However, their clinical utility has been hampered by the tumorigenic potential elicited by the residual PSCs in the differentiated cell population, the lengthy and inefficient differentiation process (Hu et al., 2010), and genomic instability (Weissbein et al., 2014). In the process of trans-differentiation, one mature somatic cell type can be converted into another functional mature or progenitor cell type without undergoing an intermediate pluripotent state by using a variety of inducers, such as transcription factors, epigenetic modifiers, and microRNAs (Moradi et al., 2014; Pournasr et al., 2011). Mature neurons have been successfully trans-differentiated from several cell sources (Ambasudhan et al., 2011; Ladewig et al., 2012; Marro et al., 2011; Vierbuchen et al., 2010). However, their inability to proliferate and survive for long periods of time in culture conditions limits their use. An alternative approach is to convert somatic cells into NSCs, which are expandable in vitro and have the potential to differentiate into major neural cell types, such as neurons, oligodendrocytes, and astrocytes.

Ectopic expression of several combinations of genes via lentiviruses with or without small molecules have been used to produce induced NSCs (iNSCs) from somatic cells (Cassady et al., 2014; Han et al., 2012a; Lujan et al., 2012; Ring et al., 2012; Thier et al., 2012; Wang et al., 2013). Most of these NSC-induction cocktails depend on the use of potentially tumorigenic pluripotency-associated factors in reprogramming or a multi-factor strategy that increases the intricacy of the approach. Hence, the use of a single reprogramming factor for generation of iNSCs may represent a more controllable, easier, and safer approach.

Here, we demonstrate that iNSCs could be generated from human fibroblasts by ectopic expression of a single neurogenic factor, zinc-finger protein 521 (*Zfp521*). Our data indicate that *Zfp521* alone is sufficient for conversion

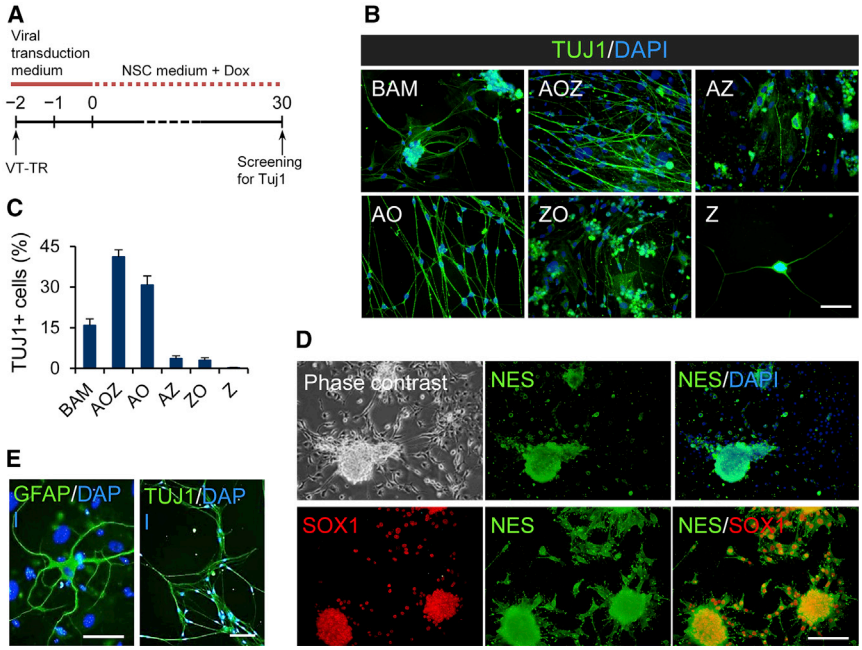

Figure 1. Reprogramming Potential of Different Combinations of Neurogenic Transcription Factors

(A) Schematic procedure for reprogramming of mouse 3T3 fibroblasts to neuronal cells by screening diverse neurogenic factors.

(B) Immunofluorescent staining for TUJ1 protein on day 30 after Dox treatment.

(C) The efficiency of neuronal reprogramming of 3T3 fibroblasts by different gene cocktails calculated 30 days after infection as the number of TUJ1-positive cells relative to the number of starting cells initially seeded. Data are shown as means ± SD of three independent experiments.

(D) Phase-contrast image of NSC-like colony emerged in *Zfp521* (Z) treatment group and immunofluorescent double-staining of these NSC-like spheres for NES (Nestin) and SOX1 on day 30 after Dox treatment.

(E) Neurons (TUJ1) and astrocytes (GFAP) differentiated from NSC-like spheres that were induced by *Zfp521*.

Nuclei in (B), (D), and (E) were counterstained with DAPI. Scale bars represent 100 μm in (B) and (E), and 200 μm in (D). See also Figure S1.

of fibroblasts into iNSCs, which may serve as an alternative and more accessible source of cells for neural cell-replacement therapies as well as in vitro disease modeling, toxicity testing, and drug development.

RESULTS

Reprogramming Potential of Different Combinations of Neurogenic Transcription Factors

We initially used murine 3T3 fibroblasts to screen for a novel reprogramming cocktail that can efficiently derive neuronal-like cells. Cells were transfected with inducible lentiviruses in six combinations of neurogenic transcription factors encoding *Math3*, *Ngn2*, *Oct6*, *Zfp521*, *Ascl1*, *Myt1l*, and *Brn2*. These neural lineage-instructive transcription factors were selected based on their key roles in normal neurogenesis (Kamiya et al., 2011; Son et al., 2011). The previously reported cocktail *Brn2-Ascl1-Myt1l* (BAM) (Vierbuchen et al., 2010) served as the positive control. For controlled ectopic expression of the transgenes, we used lentiviruses that were inducible with doxycycline (Dox). First, we confirmed that Tuj1 or other neuronal markers were not expressed in native 3T3 fibroblasts (Figure S1A) or in fibroblasts that were cultured in neural induction medium without Dox after transduction with *Zfp521*-expressing lentivirus or in cells that were transduced with empty vector and cultured in neural induction medium on day 30 after exposure to Dox (Figure S1B).

Detection of the neuronal marker Tuj1 by using immunofluorescence on day 30 after transduction with *Zfp521*-expressing lentivirus and exposure to Dox was also used as readout for successful neuronal reprogramming (Figure 1A). With this approach, we found that, in addition to the BAM group, five gene combinations (*Ascl1-Oct6-Zfp521* [AOZ]; *Ascl1-Oct6* [AO]; *Ascl1-Zfp521* [AZ]; *Zfp521-Oct6* [ZO]; and *Zfp521* alone [Z]) yielded Tuj1-positive cells with different efficiencies (Figure 1B). To estimate the conversion efficiency on day 30 of reprogramming, we determined the frequency of Tuj1-positive cells relative to the number of initially seeded 3T3 cells in three independent experiments (Vierbuchen et al., 2010). The efficiencies ranged from 0.2% ± 0.1% (Z group) to 30% ± 3.3% (AO group), and 40% ± 2.5 (AOZ group), which was even higher than in the BAM group (16% ± 2.4%; Figure 1C). This result indicates that our gene cocktails could successfully induce the neuronal phenotype in cultured fibroblasts. Unexpectedly, in the Z group, several cell spheroids emerged that were morphologically similar to spheres typically formed by wild-type NSCs, and expressed the NSC markers Nes (Nestin) and Sox1 (Figure 1D). These spheroids could also be differentiated into Tuj1- and Gfap-positive cells (Figure 1E). Therefore, *Zfp521* seemed to be capable of directly converting murine fibroblasts into NSC-like cells.