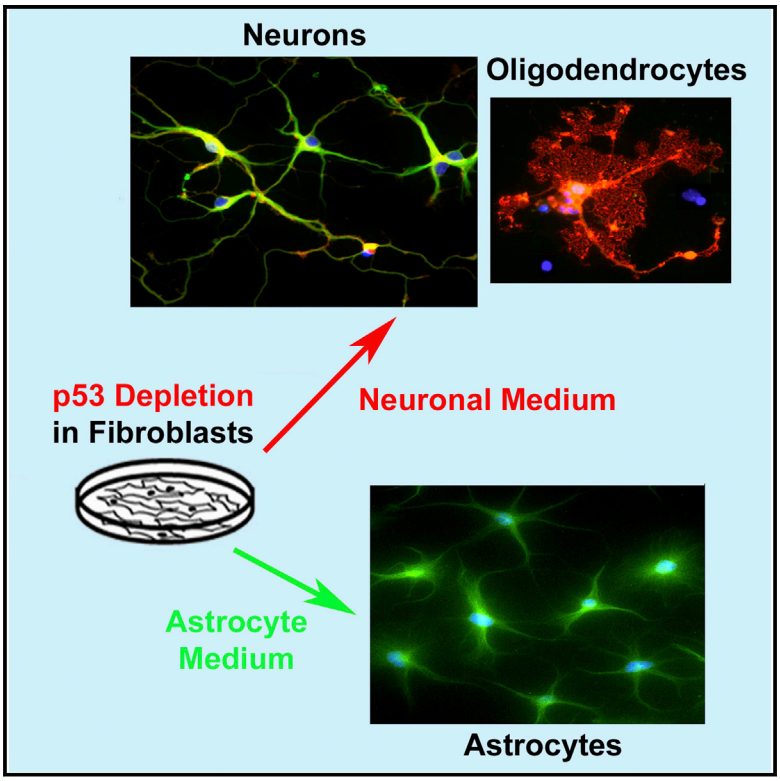
Cell Reports

Report

Conversion of Fibroblasts to Neural Cells by p53 Depletion

Graphical Abstract



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In Brief

Conversion from fibroblast to neuron has recently been successfully induced. However, the underlying mechanisms are poorly understood. Zhou et al. found that depletion of *p53* alone converted fibroblasts into three major neural lineages. This finding may help understanding reprogram mechanisms and developing cell-based replacement therapies to neurological disorders.

Highlights

- Depletion of *p53* alone converts fibroblasts into three major neural lineages
- The induced neurons are functional in vitro and in vivo
- *p53* regulates neurogenic transcription factors for fibroblast-neuron conversion
- Genome-wide transcription profile is altered during fibroblast-neuron conversion

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Conversion of Fibroblasts to Neural Cells by p53 Depletion

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SUMMARY

Conversion from fibroblasts to neurons has recently been successfully induced. However, the underlying mechanisms are poorly understood. Here, we find that depletion of p53 alone converts fibroblasts into all three major neural lineages. The induced neuronal cells express multiple neuron-specific proteins and generate action potentials and transmitter-receptor-mediated currents. Surprisingly, depletion does not affect the well-known tumorigenic p53 target, p21. Instead, knockdown of p53 upregulates neurogenic transcription factors, which in turn boosts fibroblast-neuron conversion. p53 binds the promoter of the neurogenic transcription factor Neurod2 and regulates its expression during fibroblast-neuron conversion. Furthermore, our method provides a high efficiency of conversion in late-passage fibroblasts. Genome-wide transcriptional analysis shows that the p53-deficiency-induced neurons exhibit an expression profile different from parental fibroblasts and similar to control-induced neurons. The results may help to understand and improve neural conversion mechanisms to develop robust neuron-replacement therapy strategies.

INTRODUCTION

Differentiated somatic cells have been reprogrammed to a pluripotent state by forced expression of a set of transcription factors (Takahashi et al., 2007), indicating that terminally differentiated cells can be induced to undergo cell fate change. Recent studies further demonstrated that direct conversion from fibroblast to neuron, a potential cell replacement therapy for neurological disorders, can be induced by a set of transcription factors without passing through a pluripotent state (Caiazzo et al., 2011; Vierbuchen et al., 2010; Pfisterer et al., 2011; Pang et al., 2011; Yoo et al., 2011; Ambasudhan et al., 2011). However, the mechanism underlying this conversion process remains largely unclear. As a result, a variety of combinations of transcription factors have been tried but generally with low percentages and very slow time course of conversion.

The p53 tumor suppressor reduces cancer initiation by inducing apoptosis, cell cycle, and senescence. For functions of p53 in the neural fate, it potently limits the growth of immature and mature neurons in response to a variety of stress signals. Recent studies show new roles of p53 in a wide range of processes, including neural precursor cell self-renewal, differentiation, and neuron fate decisions (Lanni et al., 2012; Hede et al., 2011; Mendrysa et al., 2011). Furthermore, p53 has been shown to inhibit reprogramming of fibroblasts to induced pluripotent stem cells (iPSCs) (Hong et al., 2009; Utikal et al., 2009; Kawamura et al., 2009), which raised a possibility that p53 might suppress fibroblast conversion to neurons. Here, we investigated whether p53 controls conversion of fibroblasts to neural cells. We found that depletion of *p53* alone could convert fibroblasts into astrocytes, oligodendrocytes, and functional neurons. Depletion of *p53* mediated this conversion by upregulating a set of neurogenic transcription factors.

RESULTS

Knockout of p53 Converts Fibroblasts into Neurons, Astrocytes, and Oligodendrocytes

We tested the possibility whether p53 might suppress fibroblast conversion to neurons by establishing a $p53^{-/-}$ cell line using zinc finger nuclease (ZFN) technology to knock out *p53* in normal human primary fibroblasts (IMR90). The *p53*-specific ZFNs are fusion proteins including the engineered zinc finger proteins that specifically bind to exon 3 of p53 genomic DNA and the nonspecific nuclease domain of restriction enzyme FokI that generates double-strand DNA cleavage. The repair of genomic DNA through the cellular process of nonhomologous end joining resulted in deletion of exon 3, creating a functional knockout of *p53*. $p53^{-/-}$ monoclonal lines were verified by quantitative PCR (qPCR), Southern and northern blot, and genomic DNA sequence (Figures S1A–S1D). The knockout was further evident