Tuning oncogenic signaling landscapes to drive efficient reprogramming to motor neurons

by

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Abstract

Direct reprogramming of patient-specific somatic cells into neurons remains limited by an incomplete understanding of how signaling pathways and gene dosage govern cell-fate transitions, and by a lack of tools that enable precise and robust gene regulation in primary cells. Using a well-defined model of direct conversion, we examined how levels of the MAPK-activating oncogene $HRAS^{G12V}$ influence reprogramming of primary fibroblasts to induced motor neurons. We show through chemogenetic tuning of MAPK signaling that a non-monotonic relationship exists between pathway activity, proliferation, and conversion efficiency. An optimal “Goldilocks” level of MAPK signaling efficiently drives cell-fate programming, whereas high levels of $HRAS^{G12V}$ induce senescence. In addition to proliferation, MAPK signaling influences conversion by regulating Ngn2 activity. We were able to eliminate the need for mutant HRAS by inducing MAPK signaling via a small molecule, PMA.

Because RAS signaling output directly controls conversion efficiency through proliferation, this system provides a functional testbed for developing and adapting synthetic biology tools for complex cellular contexts. We engineer a DNA-based promoter editing system and a compact, small-molecule-responsive RNA splicing switch to enable tunable control of RAS during long-term reprogramming processes, and identify and overcome key design bottlenecks, including transgene toxicity and viral vector architecture.

Finally, we established a high-efficiency and reproducible platform for direct conversion of primary human dermal fibroblasts into motor neurons. An optimized proliferative support cocktail and simplified transcription factor delivery strategy markedly improves conversion efficiency and consistency across multiple patient-derived primary fibroblast lines. Transcriptomic analysis confirms induction of neuronal and motor neuron programs and reveals neuron subtype-specific differences. Additionally, selective removal of dividing cells improves neuronal purity and morphological maturation.

Collectively, this work defines principles linking oncogenic signaling to cell-fate conversion, develops synthetic tools for tunable gene regulation in primary cells, and establishes a scalable human motor neuron reprogramming platform. These advances provide both mechanistic insight and practical foundations for future studies of neuronal therapies and disease modeling using directly reprogrammed human cells.

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