SYNTHETIC AND SYSTEMS BIOLOGY APPROACHES TO ENGINEER CELL FATE TRANSITIONS FOR CELL THERAPIES

By Nathan B. Wang

Cell fate reprogramming can be used to make patient-specific therapeutic cells from easily accessible donor cells for cell therapies or disease modeling. However, cell reprogramming is a time- and cost-intensive process due to low rates of reprogramming and inefficient manufacturing processes.

In my thesis, I developed systematic approaches and tools to improve reprogramming rates using the direct conversion of mouse embryonic fibroblasts to induced motor neurons (MEF to iMN) as a testbed for cell fate reprogramming. Typical synthetic biology approaches to engineering reprogramming focus on the expression and control of transcription factors. However, low rates of reprogramming obscure how transcription factor levels lead to successful or failed cell fate conversions.

To address the issue of low reprogramming rates, we developed a tailored, high-efficiency conversion system that increases the direct conversion of mouse embryonic fibroblasts to induced motor neurons (MEF to iMN) by 1,000-fold, resulting in yields >1,000% (i.e. 1 iMN for each starting MEF) and with purities of >50%. By tailoring the cocktail to increase conversion rates, we were able to examine how transcription factors act together with receptive cells states that we identify by proliferation history. By examining proliferation history and transcription factor levels simultaneously, we show that cell state—as set by proliferation history—defines how cells interpret the levels of transcription factors. Further, specific transcription factors like Brn2, can inhibit receptive cell states.

Using these insights, we then optimized the reprogramming process across molecular and systems scales by exploring process parameters such as reprogramming factor encoding and delivery. After validating iMNs through biochemical and functional assays, we show that converted iMNs can engraft with the mouse central nervous system. Altogether, this thesis shows a synthetic and systems biology approach to engineering highly efficient direct conversion processes. Through molecular and systems optimization, we developed novel approaches and tools that increase MEF to iMN conversion yields and purities by several orders of magnitude for efficient reprogramming. The systems and tools developed in this thesis enables the use of reprogrammed iMNs as a defined neural cell source for spinal cord injury mouse models in future studies.

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