MOLECULAR MECHANISMS DEFINING AND DRIVING RECEPTIVITY IN THE CONVERSION OF FIBROBLASTS TO MOTOR NEURONS

By Adam M. Beitz

Cell fate conversion processes have the potential to revolutionize drug discovery and drive the development of novel cell-based therapies, but their translational potential is limited by poor conversion rates. Layers of regulation stabilize cellular identity and prevent aberrant cell-fate transitions. Oncogenes destabilize cell states to induce cancer. We find that an oncogene cocktail also promotes cell fate conversions, enhancing conversion yield over 100-fold. To improve translationally-relevant conversion processes, we can dissect highly-efficient oncogene-mediated conversion to identify mechanisms that allow cells to convert from one cell type to another.

In my thesis, I use the model system of mouse embryonic fibroblast to motor neuron conversion to determine how oncogenic mutants of HRAS and the tumor suppressor protein p53 promote conversion. We find that cells that proliferate at high rates early in conversion attain the motor neuron identity at higher rates than cells that do not proliferate as much. We find that oncogene-induced hyperproliferation promotes conversion by inducing a receptive cell state. We isolate cells that attain high rates of proliferation and define the subcellular properties of these conversion-receptive cells. We find that receptive cells show globally-reduced transcription rates with a correlated global reduction in chromatin accessibility, mediated by epigenetic chromatin modifications. Our data shows that oncogene-induced hyperproliferation destabilizes the initial cell state and induces a receptive state with reduced transcriptional output which neuronal transcription factors may act on to successfully launch the motor neuron transcriptional state.

In the second half of my thesis, I will discuss our work to dissect the role of the p53 mutant in our oncogene-cocktail. We make a counter-intuitive finding that the interaction between mutant and native p53 enhances conversion beyond its role in promoting proliferation that is dependent on the presence of native p53. The p53 mutant induces accumulation of native p53 in a subpopulation of cells. We then developed a tool to track p53 levels in mouse embryonic fibroblasts in order to track p53 accumulation during conversion. By developing tools to isolate cells with different conversion-receptivity during oncogene-enhanced conversion, we can characterize the subcellular features that promote conversion. In my thesis, we identify oncogene-induced cell states that enable conversion that may have implications for cancer and that plasticity of cells observed in disease.

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