Abstract

CRISPR-Cas9 is a novel genome editing tool which I am using to expand the Yi lab's gene knockout repertoire. CRISPR is superior to older editing tools, such as zinc finger nucleases, since Cas9 is a generic nuclease capable of generating double stranded breaks wherever its RNA guide anneals. RNA guides, or sgRNAs, are easily engineered and can be introduced to a cell singly to cause a random disruptive mutation, or in groups, yielding multiple large deletions.

My first project focused on the deletion of the Let7 microRNA (miRNA) family in murine keratinocytes. There are 9 Let7 family members present in these cells, all acting as translational suppressors of the same target mRNAs. This redundancy implies that Let7 is crucial to cellular functioning, and it has indeed been found to act as a tumor suppressor, down regulating functions such as proliferation, cell motility, and de-differentiation. I hypothesized that increasing loss of Let7 loci would lead to progressively more tumor-like phenotypes in the cells. Phenotypic analysis of these cells has not yet been possible, but initial Let7 knockouts were successful.

Successful deletion of two Let7 clusters served as a proof of concept when a more immediately applicable use for CRISPR presented itself as part of a Down Syndrome study. The amyloid precursor protein (APP) is found on chromosome 21 and is strongly upregulated in individuals with Down Syndrome (DS). This increased protein level is thought to play a role in the early onset development of amyloid-beta plaques in the brains of those with DS, one of the hallmarks of Alzheimer's Disease. APP was therefor chosen as a target for CRISPR, not to knock it out, but to delete one copy and reduce its gene dosage to normal levels. This would allow us to assess protein and RNA levels between trisomic and disomic APP, against a background of trisomy 21. If successful, our work will serve as a proof of principle that this technique can be used to assess the dosage effect for any chromosome 21 gene. To attempt this, we need transient transfection, which is difficult to achieve with plasmids that can last in the cells for weeks. We hypothesized that brief periods of editing, and therefor incomplete knockouts, could be generated by transfecting cells with CRISPR RNAs: Cas9/EGFP mRNA, and two sgRNAs. This was attempted in both lymphoblastoid cells and HEK293T cells, but a knockout has yet to be realized.