Cell-Type-Specific Knockdown via Programmable RNA-Based Sensors

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Multicellular organisms are composed of a diverse array of specialized cell types, each defined by a characteristic transcriptomic signature. These signatures remain highly informative even under pathophysiological conditions, where normal cell identities become disrupted but still generate distinct disease-associated transcriptional states that drive malignant behavior. The differential expression of mRNA transcripts across cell types can serve as a target for precision gene regulation. However, we currently lack tools capable of selectively isolating and correcting these aberrant cell populations without affecting surrounding healthy cells. Recent advances such as single-cell RNA sequencing and spatial transcriptomics have enabled detailed detection of transcriptional states, but largely provide only fixed, observational snapshots and remain correlational. Moreover, conventional perturbation strategies typically require the generation of engineered lines, limiting flexibility, scalability, and applicability to native human tissues and dynamic disease contexts. To address these limitations, this project employs a programmable RNA-based sensor system in which a sensor RNA (sesRNA) bearing a cell-type-specific sequence hybridizes to a target mRNA transcript expressed in a defined cell population of interest. Coupling this to ADAR-mediated RNA editing enables translation of Cas13 in a cell-type-specific manner. The resulting sesRNA-Cas13 platform thereby links endogenous transcriptional states to programmable, post-transcriptional gene knockdown.

We hypothesize that sesRNA-Cas13 constructs bearing a targeting sensor sequence will selectively drive Cas13 translation and transcript knockdown exclusively in cells expressing the cognate target, with negligible off-target activity in non-expressing cells. To test this, the project will first characterize sesRNA-Cas13 performance in a single-population model. mCherry tagged HEK293 cells will be transduced via lentiviral vectors with either mCherry-targeting ($sesRNA^{mCherry}$) or non-targeting ($sesRNA^{OFF}$) Cas13 constructs, each paired with mCherry-directed guide RNAs and ECFP-tagged. Construct delivery will be confirmed by $ECFP^{+}$ fluorescence, while Cas13 expression, knockdown efficiency, and off-target leakage will be quantified by fluorescence microscopy, FACS, and immunostaining. This project will then demonstrate cell-type-specific perturbation in a heterogeneous co-culture of mCherry-expressing and wild-type HEK293 cells and transducing both populations with the $sesRNA^{mCherry}$-Cas13 construct. Selective mCherry depletion in the expressing sub-population assessed by fluorescence microscopy at 24, 48, and 72 hours and confirmed by immunostaining for Cas13– will serve as proof-of-concept for programmable, transcript-dependent gene knockdown. This framework has broad applications for understanding gene function in homeostasis, examining how malignant cells diverge from their normal counterparts, and ultimately developing cell-type-selective therapeutic strategies capable of discriminately silencing pathogenic cellular programs.