Potent transcriptional activation using CRISPRa with synthetic crRNA

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Abstract

The CRISPR-Cas system derived from Streptococcus pyogenes has been adapted to upregulate any gene in its endogenous context, enabling overexpression experiments without a need for exogenous overexpression plasmids. For CRISPR activation (CRISPRa), the guide RNA forms a complex with a nuclease-deactivated Cas9 (dCas9, D10A and H840A) which is in turn fused to transcriptional activators. The machinery then acts upstream of the transcription start sites to upregulate expression of a target gene. The ease of programming the CRISPRa system with small RNA guides is transformative for downstream genes. This demonstrates that CRISPRa using synthetic crRNAs could be used for low- or high-throughput studies of downstream signaling and pathway analysis. The downstream genes. This demonstrates that CRISPRa using synthetic crRNAs could be used for low- or high-throughput studies of downstream signaling and pathway analysis. The methods presented are broadly applicable as a strategy to upregulate any gene including systematic functional gene analysis in an arrayed screening format.

Efficient transcriptional gene activation with synthetic crRNA in dCas9-VPR stable cells

HEK293T, U2OS, MCF 10A, and NH/3T3 cells stably expressing dCas9-VPR were transfected with synthetic crRNA:tracrRNA (25 nM) targeting POU5F1 and TTN using DharmaFECT transfection reagents. K-562 cells were electroporated with synthetic crRNA:tracrRNA (400 nM) targeting POU5F1 and TTN. Cells were harvested 72 hours post-transfection and the relative gene expression was assessed using RT-qPCR. The relative expression of each gene was calculated using the ΔΔCt method with GAPDH as the reference gene, and normalized to a non-targeting control. Varying levels of gene activation of POU5F1 and TTN are observed in all cell lines compared to the endogenous gene expression level.

Pooling of crRNAs can enhance transcriptional activation

U2OS cells stably expressing dCas9-VPR were transfected with synthetic crRNA:tracrRNA targeting EGFR or POU5F1 (25 nM total concentration) using DharmaFECT 4 transfection reagent. The crRNAs were used either individually or pooled (at a total concentration of 25 nM). The pooled crRNAs demonstrate gene up-regulation activity as good or better than the most active single crRNA. The ability to easily pool crRNAs is an advantage of utilizing chemically synthesized crRNA:tracrRNA.

Immunofluorescence analysis indicates effective gene activation

U2OS-dCas9-VPR stable cells were transfected with a pool of four synthetic crRNAs targeting EGFR or POUSF1 (25 nM total concentration) using DharmaFECT 4 transfection reagent. 72 hours post-transfection, cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. Cells were stained with Hoechst 33342, primary antibodies targeting the gene of interest, and secondary antibodies conjugated to DyLight 550.

Conclusions

- Potent up-regulation of target genes with CRISPRa can be achieved with chemically synthesized crRNA:tracrRNA enabling gain-of-function studies.
- Advantages of synthetic crRNA compared to plasmid-based sgRNA for CRISPRa include the ease of testing multiple target sites per gene, easily pooling multiple sequences for increased gene activation effect, and activation of multiple genes at the same time.
- CRISPRa with synthetic crRNA can be effective in studying the downstream effects of target gene activation.