Abstract

Gene editing has the potential to revolutionize the treatment of many genetic diseases and several in vivo gene editing therapies have recently entered the clinic. To increase their safety and efficacy, precise spatial and temporal on-target editing with minimal off-target editing is highly desirable and will require tight intrinsic or extrinsic control of the gene editor. To this end, we have developed small drug-responsive domains (DRDs) as part of our cytoDRiVE® platform that can be fused to Cas9 nuclease. This enables precise control of its expression and activity by exposure to a clinically approved small molecule activating drug. The small size of these DRDs requires minimal vector space and will allow delivery of DRD-Cas9 and guide RNA in a single AAV vector. We show that the DRD-Cas9 fusions have extremely low off-target editing activity in the absence of drug which increases to near constitutive levels of activity upon treatment with activating drug for a panel of guide RNA targets. In addition, we show that fusion of DRDs to Cas9 does not affect target specificity or repair patterns of the nuclease. This level of control demonstrated in vitro by our cytoDRiVE® platform supports potential clinical utility in cell and gene therapy, where tight regulation of DRD-Cas9 would give the physician control of Cas9 activity in the patient. Pausing or stopping administration of the activating drug once edits have been made could limit off-target editing activity and reduce the risk of genotoxicity.

cytoDRiVE® technology

DRDs tightly regulate Cas9 on-target editing activity

Conclusions

- We have identified novel small FKBP13 derived Drug Responsive Domains (DRDs) regulated by an FDA approved activating drug using a high throughput screen method.
- DRD-Cas9 maintained low OFF-state activity for 14 days in the absence of activating drug and achieved high level of ON-state activity with drug treatment.
- DRD-Cas9 in the OFF-state results in reduced off-target activity and in ON-state results in similar off-target activity as compared to constitutive Cas9.
- Treatment with activating drug resulted in increase in DRD-Cas9 protein expression within 24 hours of treatment and decrease in protein expression without further treatment to demonstrate successful drug dependent expression of DRD-Cas9 protein which results in tight control of Cas9 editing activity.

Figure 1. Overview of regulation of therapeutic proteins with cytoDRiVE® technology. Drug responsive domains (DRDs) are fused to proteins of interest (POIs) containing membrane, transmembrane, or intracellular domains to enable regulation of the protein in a cell type specific manner. This enables tight control of the therapeutic protein at both the transcriptional and translational level.

Figure 2. CytoDRiVE® platform enables precise control of Cas9 expression and activity based on cellular drug concentrations. (A) Stable integration of target 1 and 2 as measured by GFP reporter expression in 293T cells treated with 0, 0.2, 3, and 10 µM Tacrolimus. (B) Indel activity results on day 7 and day 14 as measured by DRD1-Cas9 and DRD2-Cas9 expression in 293T cells treated with 0, 0.2, 3, and 10 µM Tacrolimus. (C) Target editing activity results on day 7 and day 14 as measured by DRD1-Cas9 and DRD2-Cas9 expression in 293T cells treated with 0, 0.2, 3, and 10 µM Tacrolimus.

Figure 3. Novel small FKBP13 derived DRDs were identified using a high throughput screen. Selection was performed using the CRISPR/Cas9 system and Drug Responsive Domain (DRD) regulating expression of Cas9. A pooled screen was performed using DRD1-Cas9 and DRD2-Cas9 expression in two or three dose groups in a single AAV vector. After screening, top scoring DRDs were tested in triplicate in both the OFF-state and the ON-state for target 1 and 2.

Figure 4. On-target and Off-target Cas9 activity was determined by robust reporter gene cassette for Cas9. Protein was expressed using the cytoDRiVE® platform in two different dose groups in a single AAV vector. Proteins were tested in triplicate for both the OFF-state and the ON-state for target 1 and 2.

Figure 5. Off-state DRD lower Cas9 off-target editing activity. (A) Reporter expression was measured by luciferase reporter gene cassette in the OFF-state. (B) Reporter expression was measured by luciferase reporter gene cassette in the ON-state.