Design and Assembly of CRISPR/Cas9-Based Virus-Like Particles for Orthogonal and Programmable Genetic Engineering in Mammalian Cells

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Targeted genetic engineering with CRISPR-Cas9 endonucleases is an exciting development with many potential applications in science and medicine. However, a primary challenge in realizing CRISPR's full potential in therapeutics lies in its intracellular delivery. No optimal platform exists yet for the safe, adaptable, and effective delivery of interchangeable Cas9 systems to cells within the body, limiting the technology's utility. Addressing this, guide RNA-programmed virus-like particles (VLPs) are designed to induce gene-specific knockout and upregulation in multiple targets. Engineered endonucleases tandemly incorporate directly as proteins into self-assembling viral structures. Accordingly, transduction and capsid disassembly in target cells mediate transient and modular delivery of various Cas9 proteins. Towards four VLPs each demonstrating either transgene activation or knockout with two different sgRNA constructs, 14 classes were constructed and tested, gradually determining optimal VLP design from an initial Gag-Pol-Cre framework. Final designs employed a CRISPR tandem-linking system, in which VLPs interchangeably packaged Cas9 and dCas9-VPR along with exchangeable sgRNAs through noncovalent protein-RNA interactions. VLP efficacy was analyzed with flow cytometry and luciferase bioluminescence assays of transduced TRE-GFP and TRE-GLuc genetic circuits in reporter cells, revealing at least three-fourths population expression loss and twofold expression gain in 50,000 cell populations compared to transgene transfections alone. These findings validate direct Cas9 protein delivery via viral transduction, demonstrating a new platform for the general delivery of CRISPR-based genetic therapies.

Awards Won:

First Award of $5,000