Viral and non-viral vectors for the delivery of genome editing tools to mammalian cells

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While the discovery of CRISPR-Cas systems and their application to mammalian cells has rapidly expanded genome editing tools, their medical implementation remains constrained. A key obstacle is the scarcity of suitable delivery tools for Cas nucleases and their sgRNA, particularly for advanced applications. Current adeno-associated viral vectors, being limited in size, struggle to accommodate both Cas and sgRNA. Addressing this limitation, liposomes and herpes viral (HSV) vectors emerge as promising alternatives, capable of overcoming size constraints and adaptable for in vitro, ex vivo, and in vivo applications.

This study focuses on leveraging proprietary liposomes for Cas9 and sgRNA delivery to mammalian cells. Assessment involves the delivery of Cas9 RNPs encapsulated in liposomes, with Cas9-GFP fusion facilitating the visualization of protein localization in various cells. Variable Cas9 concentrations in liposomes and diverse incubation times are tested to optimize delivery efficiency. Additionally, we explore the potential of proprietary liposomes to traverse the blood-brain barrier using an in vitro BBB model.

The second delivery approach involves HSV, targeting more intricate genome editing tools, such as prime-editors. A singular HSV vector, encompassing all prime-editor elements, was constructed using an extensible mammalian modular kit. Validation of this designed HSV vector was performed on an established reporter cell line within the laboratory, with the objective of editing genome-encoded GFP to BFP. These endeavors underscore the potential of liposomes and HSV vectors as robust delivery platforms for advancing CRISPR/Cas-based therapies, bringing us closer to overcoming existing barriers in genome editing applications.