Tools and technologies for inducible and reversible packaging of crispr/cas9 ribonucleoprotein complexes and their targeted delivery by biological nanoparticles

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INTRODUCTION

CRISPR/Cas9 systems enable precise manipulations with the genome which can be used for developing novel therapeutic approaches to treat hereditary disorders, cancer, combat viral infections etc. Complexes of Cas9 protein and single-guide RNA (sgRNA) (ribonucleoprotein complexes – RNPs) represent the most potent and specific approach for the use of gene editing technologies. However, delivering CRISPR/Cas9 RNPs in vivo is the major challenge and currently represents the biggest obstacle for moving CRISPR/Cas9 technologies into clinical trials.

AIM

To develop a new method for inducible and reversible packaging of CRISPR/Cas9 RNPs and test its antiviral activity at hepatitis B virus (HBV) in vitro models.

METHODOLOGY

Plasmids encoding CRISPR/Cas9 inducible packaging systems and functionalization systems were cloned using Gibson assembly. For induction of CRISPR/Cas9 packaging, HEK293T cells were grown at LED-illuminated plates. Functionality of packaging system was tested by fluorescent microscopy. Produced nanoparticles were characterized by DLS. Packaging of Cas9 protein was measured by FACS with conjugated microspheres. sgRNA packaging was confirmed by PCR analysis of produced NPs. Delivery of Cas9 protein to target cells was confirmed by confocal imaging. Anti-HBV activity was measured by secreted HBsAg. Functionalization was analyzed by in vitro uptake assay using FACS.

RESULTS

Here, we present the development of inducible packaging and delivery of CRISPR/Cas9 ribonucleoproteins inside biological nanoparticles. We also demonstrate successful intracellular delivery, anti-HBV activity and functionalization of NPs for liver-specific delivery.

CONCLUSION

Delivering CRISPR/Cas9 ribonucleoprotein complexes is a major challenge of gene editing. Biological NPs are highly biocompatible and can be safely used to deliver therapeutic cargo. In this study, for the first time, we demonstrate the means for delivering CRISPR/Cas9 ribonucleoprotein complexes and indicate their successful use for targeting HBV.

ACKNOWLEDGEMENTS

This study was supported by RSF grant № 20-15-00373.

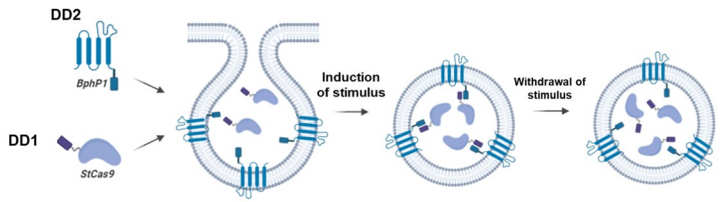

Figure 1. Schematics of CRISPR/Cas9 packaging system into biological NPs.

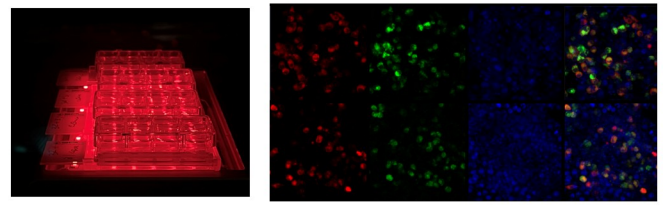

Figure 2. Light-inducible packaging of CRISPR/Cas9. (left) LED illumination of NP-producing cells. (right) induced distribution of StCas9 protein (red) into the packaging pathway (green). Cell nuclei are counterstained with Hoechst33342.

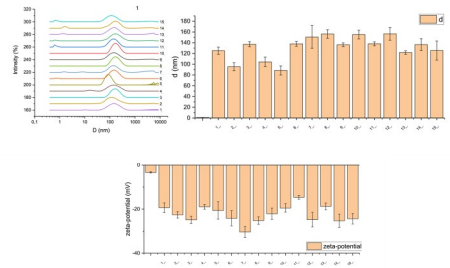

Figure 3. Characterization of NPs. CRISPR/Cas9-loaded and functionalized nanoparticles were characterized by DLS (distribution by size, mean size, zeta-potential).

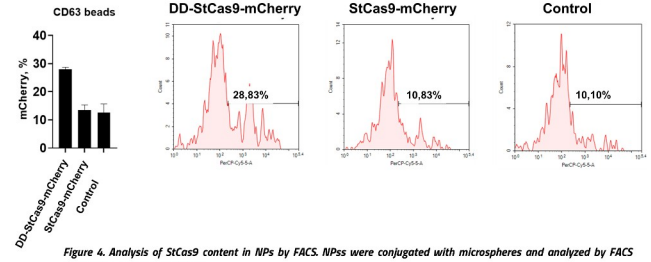

Figure 4. Analysis of StCas9 content in NPs by FACS. NPss were conjugated with microspheres and analyzed by FACS analysis. L – induction by light.

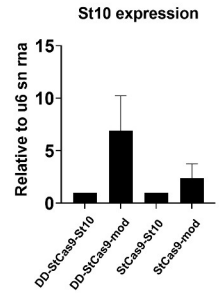

Figure 5. Induced packaging of sgRNA.

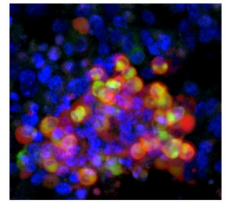

Figure 6. Delivery of CRISPR/Cas9 into cells by NPs. StCas9 protein (red) delivered by NPs (green). Nuclei are counterstained by Hoechst33342.

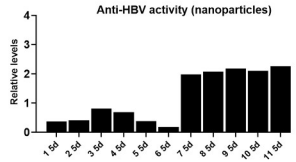

Figure 7. Anti-HBV activity of NPs loaded (1-6) and not loaded (7-11) with CRISPR/Cas9. Anti-HBV activity was measured by secreted HBsAg.

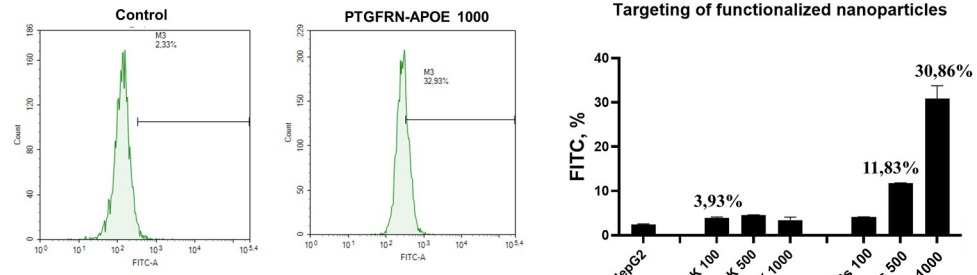

Figure 8. Targeting of HepG2 cells by functionalized NPs. (left) FACS histograms of HepG2 cells treated with NPs. (right) In vitro NPs uptake by HepG2 cells upon treatment with control nanoparticles (K) and functionalized nanoparticles (Funct-NPs). 100, 500, 1000 – added NPs by protein content.