UTILITY OF SELF-DESTRUCTING CRISPR/CAS SYSTEMS FOR TARGETED GENOME EDITING IN THE RETINA

Purpose: The CRISPR/Cas system is used by bacteria to counter viral intrusion and recently has been adapted to allow efficient editing of the mammalian nuclear genome. With a viral delivery system, active Cas9 will be maintained in the neurosensory retina for an extended period of time. Consequently, CRISPR/Cas genotoxicity is a significant consideration in clinical applications and, regardless of fidelity, it is foreseen that the overall chances of eventually cutting off-target sites which result in cytotoxicity, would accumulate with time. To address this issue, we have rationally designed and tested sgRNAs which target and disrupt SpCas9 itself.

Methods: Rather than initially focus on a disease-specific gene, we used yellow fluorescent protein (YFP)-expressing transgenic mice to investigate the efficacy of self-destructing “kamikaze” CRISPR/Cas system. Four different guide RNAs (sgRNAs) were designed to target SpCas9 and after in vivo validation, selected sgRNAs were cloned into the pX552-YFP sgRNA to generate the YFP targeting “kamikaze-CRISPR/Cas” vector. The editing efficacy of “YFP targeting kamikaze-CRISPR/Cas” AAV2 constructs were validated in vitro, before intravitreal injection.

Results: After initial expression, our self-destructing “kamikaze-CRISPR” plasmids, cease to produce functional Cas9. Marked reduction of spCas9 protein concentration (~90% at day 2) as well as YFP expression (66%~80% at day 10) were found following the transfection of YFP targeting “kamikaze-CRISPR/Cas” vector.

Conclusions: Our results suggest that our novel self-destructing kamikaze CRISPR/Cas system could be used as a safe and robust tool for gene editing. Ongoing work will validate efficacy and off-target profile of this system in vivo.