Title : Rational design of an enhanced genome editing protein for therapeutic gene correction and diagnostics

Summary:

RNA-guided, programmable CRISPR-Cas9 nucleases is a facile system heralding a new dawn in the field of therapeutic gene correction and disease detection. Safe therapeutic gene correction can only be accomplished if the Cas9 has high on-target editing efficiency and minimal off-target activity. The off-targeting effect of the most widely used system of Cas9 from *Streptococcus pyogenes* (SpCas9) and the low editing efficacy of its engineered derivatives raise serious concern on its utility as a therapeutic modality. Recently, we reported a Cas9 from *Francisella novicida* which has negligible affinity towards off-target DNA substrate thereby giving specificity in DNA interrogation. However, its low cellular targeting efficiency in the human genome is limiting its therapeutic application. We envisioned that enhancing the functional activity of FnCas9 protein might circumvent this issue. Thus, we set out to rationally engineer this FnCas9 protein guided by structure to enhance its kinetic activity. We hypothesized that stabilizing the PAM duplex-binding region of FnCas9 by introducing base non-specific interactions might improve its activity while keeping the intrinsic specificity unaltered. To this end, we generated 50 different protein variants and screened for kinetic enhancement by in-vitro cleavage assay. We discovered a subset of variants (hereafter enFnCas9) that showed around 2 fold higher cleavage kinetics compared to the wild-type protein (WTFnCas9). We discovered a subset of variants showed improved DNA binding affinity over WTFCas9 and also exhibiting PAM alterations from canonical NGG PAM to NGR/NRG PAM thereby broadening the accessibility across human genomic sites. Recently, our group has developed CRISPR diagnostic (CRISPRDx) platforms, FnCas9 Editor Linked Uniform Detection Assay (FELUDA) and Rapid Variant Assay (RAY) by harnessing the single mismatch sensitivity of FnCas9 for detection of SNPs, SARS-CoV-2, SARS-CoV-2 variants of concerns (VOCs). We found that enFnCas9 variants were able to distinguish single mismatches in the target with a much higher resolution of discrimination than WTFnCas9 and non-NGG PAM targeting improved the detection spectrum. Finally, we showed that one of the enFnCas9 variants, en1Fn showed highly improved on-target cellular genome editing across different human cell lines over WTFnCas9 while keeping DNA interrogation highly specific.

Taken together, we developed a robust genome editing and diagnostics platform by engineered FnCas9 variants.

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