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Genome-wide Specificities Comparison of ZFN, TALEN and High-fidelity CRISPR in SiHa Cell Line

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Background: Zinc-finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN) and clustered regulatory interspaced short palindromic repeat (CRISPR) RNA-guided nuclease are three types of programmable site-specific genome editing tools with a broad range of research and clinical applications. However, the knowledge of their effectiveness and genome-wide off-target cleavage activities has been lacking.

Methods: In this study, we chose three genes and designed three different sites for the same gene targeted by ZFN, TALEN and High-fidelity CRISPR (CRISPR-HF) separately. After evaluating the editing efficiency by T7E1 assay, we used the unbiased, genome-wide and sensitive DSB detection approach–-GUIDE-seq to compare their on-target and off-target profiles for total 9 different nucleases in SiHa cell line.

Results: The on-target efficiency of CRISPR-HFs was significantly higher than TALENs and ZFNs. Specifically, the average on-target efficiency of CRISPR-HF was 2.2-fold that of TALEN and 11.6-fold that of ZFN. Meanwhile, CRISPR-HFs induced less potential off-target cleavage sites (0, 1, 1, respectively) than TALENs (10, 13, 6, respectively) and ZFNs (9265, 2359, 247 respectively). However, the number of confident off-target positions was lowest in TALENs (0, 0, 0 respectively) followed by CRISPR-HFs (0, 1, 1 respectively) and ZFNs (1, 3, 0 respectively).

Conclusion: Our experiments suggest that CRISPR-HF outperformed ZFN and TALEN in cleavage efficiency and was not inferior to TALEN in off-target performance, which may serve as a promising choice in future genome-editing application.
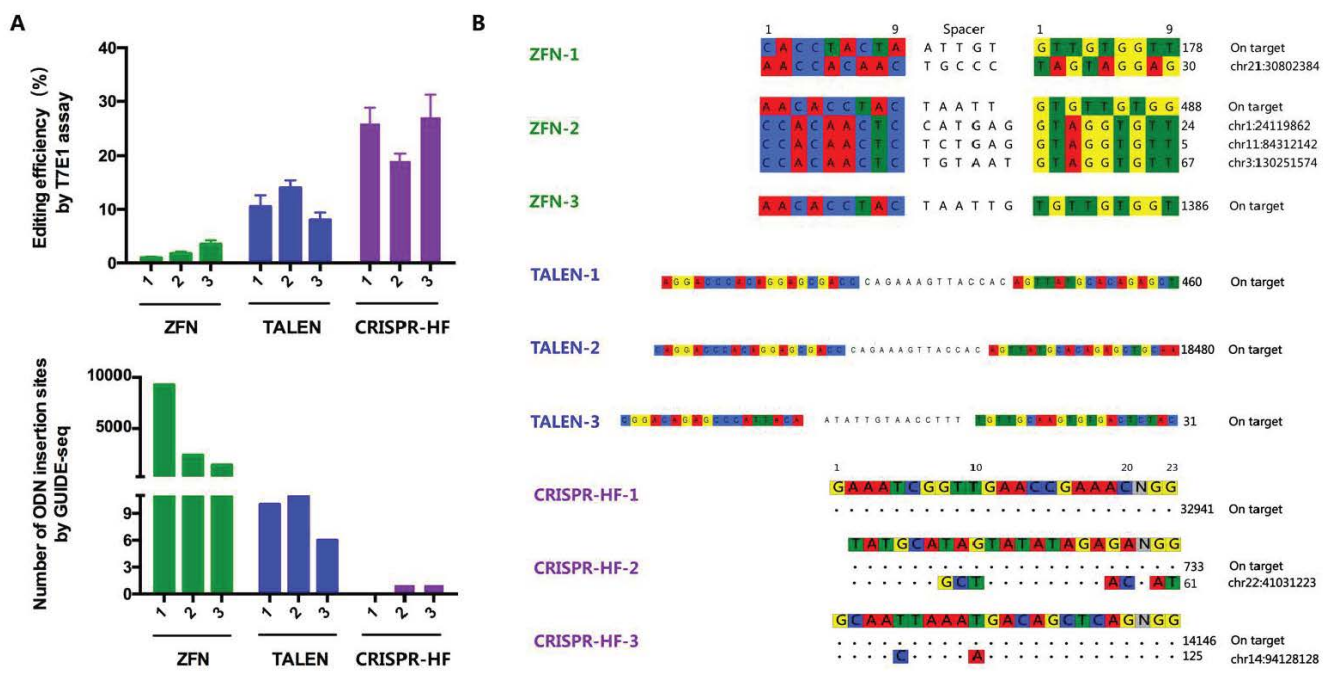
Biography:

Weiwen Fan is a PhD student. Presently working under the supervision of Zheng Hu (Doctoral Supervisor, Chief Special Scientist of the Ministry of Science and Technology, Young Top Talents in Ten-thousand Talents Program).

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