**RNA-INDEPENDENT AND METAL ION-DEPENDENT DNA NUCLEASES OF CRISPR-CAS12A**

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CRISPR-Cas12a is recognized as a promising tool for genome editing in addition to the well-studied CRISPR-Cas9. Cas12a systems use a single CRISPR RNA (crRNA) molecule and a single catalytic domain RuvC for guided double-strand DNA (dsDNA) cleavage. However, this RNA-guided DNA binding not only targets specific dsDNA sequences, but also unleashes indiscriminate single-strand DNA (ssDNA) cleavage. In addition, we previously observed that *Acidaminococcus sp*. Cas12a (AsCas12a) mediates strong ssDNase activity in divalent magnesium ions (Mg2+)-containing environment, while *Francisella novicida* Cas12a (FnCas12a) does not. To fully understand the substrate specificity and DNA cleavage pattern in the absence of crRNA, we detected the DNA substrate cleavage activity using gel electrophoresis after co-incubation with Cas12a orthologs in a buffer containing divalent metal ions. Specifically, we focused on the impacts of Cas12a orthologs (AsCas12a, LbCas12a or FnCas12a), substrate specificity (ssDNA or dsDNA; linear or circular), metal ion types (Mg2+, Mn2+, Co2+, Ca2+, Cu2+, Ni2+, and Zn2+) and concentration (0.2-10 mM), and the time course of DNA degradation (1-60 min). We also investigated the inhibition of the Cas12a-mediated DNase activity by incorporating anti-Cas12a phosphorothioate modified DNA (psDNA) to the reaction at different concentration (20-200 nM). The results demonstrated that, in spite of sharing similar sequences and functional domains, the Cas12a orthologs differ in their preference of divalent metal ions when unleashing indiscriminate DNase activities. AsCas12a and LbCas12a showed a predominant preference for cleaving both linearized and circular ssDNA in the presence of 10 mM Mg2+ and Mn2+, respectively. In addition, AsCas12a and FnCas12a caused substantial dsDNA cleavage in the presence of 10mM Mn2+, regardless of the dsDNA’s form. Importantly, these DNase activities can be inhibited by anti-Cas12a ps-DNA oligonucleotide in a sequence-independent and concentration-dependent manner, showing diminished ssDNase activity of AsCas12a, LbCas12a, and FnCas12a on ssDNA and partial inhibitory profile on dsDNA. Thus, anti-Cas12a ps-DNA represents an effective approach to regulate the DNase activities of Cas12a. Overall, ssDNase activity of the Cas12a orthologs uncovered a distinct approach for DNA cleavage compared to RNA-guided endonuclease, and provided insights into potential biological and therapeutic applications.

Reference

1. Li, B.; Yan, J.; Zhang, Y.; Li, W.; Zeng, C.; Zhao, W.; Hou, X.; Zhang, C.; Dong, Y. CRISPR-Cas12a Possesses Unconventional DNase Activity That Can Be Inactivated by Synthetic Oligonucleotides. *Molecular Therapy - Nucleic Acids* **2020**, *19*, 1043–1052.