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Original research

Temperature effect on CRISPR-Cas9 mediated genome editing

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ABSTRACT

Zinc-finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR-Cas9) are the most commonly used genome editing tools. Previous studies demonstrated that hypothermia treatment increased the mutation rates induced by ZFNs and TALENs in mammalian cells. Here, we characterize the effect of different culture temperatures on CRISPR-Cas9 mediated genome editing and find that the genome editing efficiency of CRISPR-Cas9 is significantly hampered by hypothermia treatment, unlike ZFN and TALEN. In addition, hyperthermia culture condition enhances genome editing by CRISPR-Cas9 in some cell lines, due to the higher enzyme activity and sgRNA expression level at higher temperature. Our study has implications on CRISPR-Cas9 applications in a broad spectrum of species, many of which do not live at 37°C.

1. Introduction

Zinc-finger nuclease (ZFN) (Urnov et al., 2010), transcription activator-like effector nuclease (TALEN) (Bogdanove and Voytas, 2011), and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR-Cas9) (Doudna and Charpentier, 2014; Hsu et al., 2014) are invaluable tools for genome editing. When introduced into cells, these nucleases can induce DNA double-strand breaks (DSBs) at specified genomic loci. DSBs are repaired either by non-homologous end joining (NHEJ), or by homology-directed repair (HDR), leading to various types of genetic modifications (Wiles et al., 2015). Many efforts have been made to improve the performance of these nucleases. One simple but robust method is to culture the cells in transient hypothermia conditions (30°C), which can significantly enhance ZFN- and TALEN-mediated genome editing efficiency (Doyon et al., 2010; Carlson et al., 2012). However, how temperature affects the efficacy of the CRISPR-Cas9 system has not been characterized.

Here, we characterize the effect of temperature on CRISPR-Cas9 mediated genome editing and show that the genome editing efficiency of CRISPR-Cas9 is substantially hampered by hypothermia treatment, while hyperthermia (39°C) culture can enhance the efficiency in some cell lines. We also analyze the underlying mechanisms of this phenomenon.

2. Results

2.1. Comparing ZFN, TALEN and CRISPR-Cas9 genome editing efficiency at the AAVS1 locus at different temperatures

First, we compared the genome editing efficiency of ZFN, TALEN and CRISPR-Cas9 systems at the *AAVS1* (adeno-associated virus integration site 1) locus at different culture temperatures. Based on the previously published ZFN and TALEN target sites (Hockemeyer et al., 2009, 2011), we designed the CRISPR single guide RNA (sgRNA) so that all three types of nucleases would generate DNA DSBs at very similar sites at the *AAVS1* locus (Fig. 1A and Table S1). K562 cells were grown to the optimal density at 37°C, and then electroporated with plasmids encoding each nuclease system along with pMAX-GFP plasmid (Lonza, Germany) using Lonza 4D-nucleofector X. After nucleofection, cells were equally divided into four parts, and cultured at 30°C, 33°C, 37°C, and 39°C independently. Three days later, samples were collected for fluorescence activated cell sorting (FACS) analysis. The transfection efficiency was consistent at different temperatures, while higher temperature led

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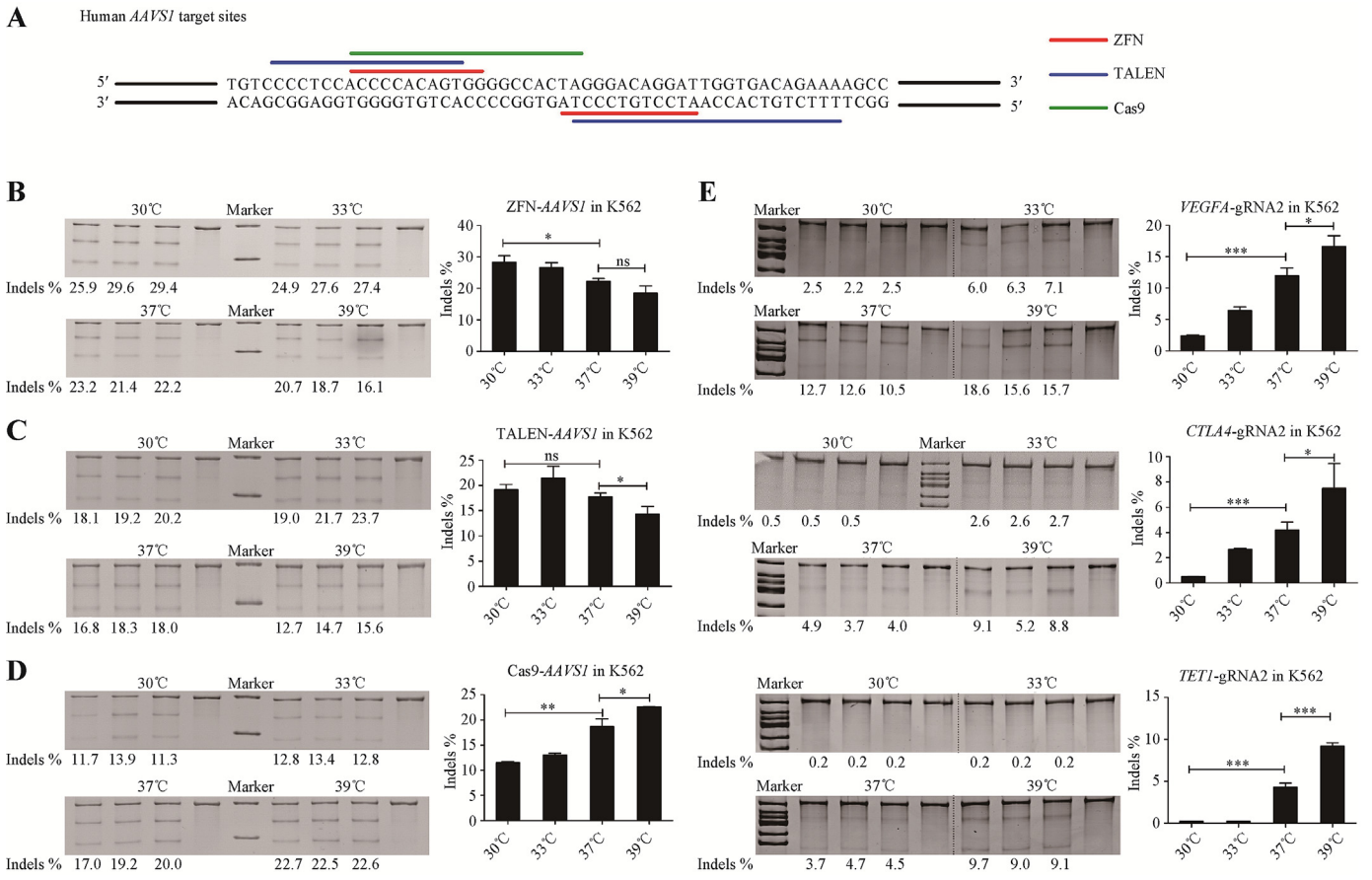

Fig. 1. The temperature effect on ZFN, TALEN and CRISPR-Cas9 mediated genome editing. **A**: Schematic of ZFN, TALEN and CRISPR target sites at the human *AAVS1* locus. Surveyor assay to determine the editing efficiency of ZFN (**B**), TALEN (**C**), and CRISPR-Cas9 (**D**) at human *AAVS1* locus. **E**: Surveyor assay to determine the CRISPR-Cas9 editing efficiency at *VEGFA*, *CTLA4*, and *TET1* loci in K562 cells. Experiments were performed in three biological replicates, and error bars indicate SD. On the gel, each temperature had four samples: the left three are experimental samples, and the right one is wild-type control. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, no significance.

to higher cell number (Fig. S1).

Next, we utilized the Surveyor assay (Guschin et al., 2010) to analyze genome editing efficiency. As previously reported (Doyon et al., 2010; Carlson et al., 2012), ZFN treated samples cultured at 30°C and 33°C had higher genome editing efficiency than samples cultured at 37°C (Fig. 1B). The temperature effect in TALEN treated samples was similar but not as evident (Fig. 1C). Surprisingly, we found that the CRISPR-Cas9 system performed in an opposite manner, with higher culture temperature leading to a higher editing efficiency (Fig. 1D). Gene editing efficiency improved from 11.5% at 30°C to 18.7% at 37°C, suggesting a lower temperature impeded the CRISPR-Cas9 activity. Moreover, 39°C culture conditions resulted in editing efficiency higher than 37°C culture (Fig. 1D).

2.2. Evaluating multiple target sites in K562 cells

To verify whether the same effects can be observed at other genomic loci, we tested nine different sgRNAs targeting different genes in K562 cells (Table S1). The overall effect of culture temperature on genome editing efficiency is similar to the *AAVS1* results (Figs. 1E and S2). Notably, several sgRNAs leading to robust gene editing at 37°C were barely functional at 30°C. Meanwhile, 39°C culture led to significantly higher indel (insertions or deletions) frequency compared to 37°C culture at several loci (Figs. 1E and S2).

To test whether the plasmid dosage contributes to the temperature effect on genome editing efficiency, we tested two dosages of plasmids (2 μg and 5 μg) encoding either TALEN or CRISPR-Cas9 systems, targeting *OCT4* and *MECP2* loci, and cultured the cells at different temperatures. For both systems at each temperature, the overall editing efficiency increased with increasing plasmid amount. The temperature effect was similar using either dosage, with higher culture temperature leading to more efficient CRISPR-Cas9 activity and less robust TALEN mediated gene editing (Fig. S3).

2.3. Evaluating multiple cell lines

To verify our findings in other cell types, we targeted multiple genes in several commonly used cell lines including human embryonic stem cells (ESCs). In accordance with the results acquired from K562 cells, we found that CRISPR-Cas9 genome editing efficiencies increased at higher temperature in Jurkat cells. The indel frequencies at the *AAVS1* and *B2M* loci at 39°C were significantly higher than those at 37°C culture (Fig. 2A). While we did not observe the efficiency improvement of the 39°C culture in the other cell lines we tested, we observed a consistent editing efficiency improvement from 30°C to 37°C (Figs. 2B and S4).