Enhancing genome editing efficiency in goldfish (Carassius auratus) through utilization of CRISPR-Cas12a (Cpf1) temperature dependency

Huijuan Li , Lili Liu , Xiaowen Wang , Rong Zhang , Hua Zhu *

Beijing Key Laboratory of Fishery Biotechnology, Fisheries Science Institute, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China

Corresponding author. E-mail address: zhuhua@bjfishery.com (H. Zhu).

Abstract

The CRISPR/Cas technology has demonstrated revolutionary potential across various fields, including agricul-ture, medicine, and food safety detection. However, the utility of CRISPR/Cas12a, a particularly promising gene- editing tool, is constrained by its temperature sensitivity, limiting its application in low-temperature environ-ments. In this study, we developed a gene-editing technique based on the CRISPR/Cas12a system in the poiki-lothermic species goldfish Carassius auratus. We systematically evaluated the editing efficiencies of LbCas12a and AsCas12a on the tyrosinase (*tyr*) gene under varying temperature conditions. Our results revealed a pronounced temperature dependence of Cas12a, with elevated temperatures markedly enhancing its editing activity, particularly for AsCas12a. A brief one-hour high-temperature treatment was sufficient to achieve effective gene disruption, underscoring CRISPR/Cas12a as a rapid and efficient gene-editing tool. Temperature was utilized as a conditional trigger for Cas12a-mediated gene knockout, enabling precise modulation of gene disruption at specific embryonic developmental stages. These findings indicated that CRISPR/Cas12a represented a viable alternative to the widely utilized CRISPR/Cas9 system and could be applied in conjunction, thereby expanding the potential applications of gene-editing technologies.

Results

Investigation of temperature effects on Cas12a editing efficiency

To elucidate the effects of elevated emperatures on embryonic development, we investigated hatching rates at five distinct temperatures: 15.5 ◦C, 18.5 ◦C, 23.5 ◦C, 26.5 ◦C, and 29.5 ◦C. The effectiveness of crRNAs at different temperatures was analyzed by sanger sequencing. The results revealed that LbcrRNA1, LbcrRNA2, and LbcrRNA3 effectively directed LbCas12a-mediated mutagenesis, as indicated by the presence of clear multiple peaks proximal to the PAM sites of tyrA and tyrB at five temperatures. The effectiveness of crRNAs at different temperatures was analyzed by sanger sequencing. The results revealed that LbcrRNA1, LbcrRNA2, and LbcrRNA3 effectively directed LbCas12a-mediated mutagenesis, as indicated by the presence of clear multiple peaks proximal to the PAM sites of tyrA and tyrB at five temperatures (Fig. 1).

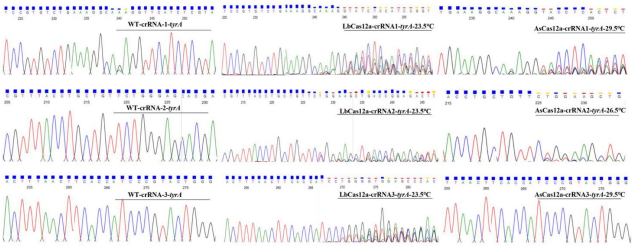

Fig.1. Sanger sequencing analysis of PCR-amplified DNA from injected embryos.

Investigation of temperature effects on Cas12a phenotype efficiency

Our findings demonstrated a notable increase in the incidence of albino phenotypes with rising temperatures for both LbCas12a and AsCas12a systems, with AsCas12a exhibiting a more pronounced temperature sensitivity (Fig. 2). At 15 ◦C, both LbcrRNA1/LbCas12a and LbcrRNA2/LbCas12a exhibited low knockout efficiencies for tyr, with only approximately 4 % of the individuals displaying albinism. As the temperature increased, the editing efficiencies of both LbcrRNA1/LbCas12a and LbcrRNA2/ LbCas12a rose to approximately 70 % at 18 ◦C. However, the proportion of mosaic individuals was significantly higher, with LbcrRNA1/ LbCas12a resulting in 54.69 % mosaic individuals and LbcrRNA2/ LbCas12a yielding 66.86 %. In contrast, LbcrRNA3/LbCas12a displayed a mosaic proportion of 8.96 % at 15 ◦C, with 91.04 % of individuals exhibiting a wild-type phenotype. At 18 ◦C, 2.74 % of individuals showed albinism, and the proportion of mosaic individuals was 8.22 %. The proportion of albino individuals increased with temperature, reaching a peak of 80.88 % at 29.5 ◦C. In contrast, the prevalence of mosaic phenotypes decreased as the temperature rose.

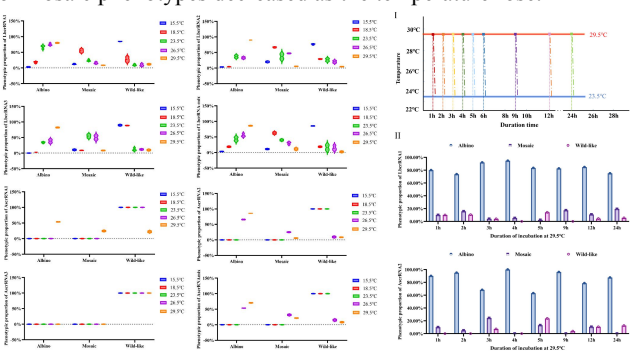

Fig. 2. The phenotypic proportion of LbCas12a/LbcrRNAs and AsCas12a/AscrRNAs at different temperature.

For AscrRNA1/AsCas12a, 54.1 % of the subjects displayed an albino phenotype, while 25.9 % exhibited a mosaic phenotype at 29.5 ◦C. Similarly, with AscrRNA2/AsCas12a, no mutant phenotypes were observed at 23.5 ◦C. At 26.5 ◦C, the frequencies of albino and mosaic phenotypes were 66.4 % and 25.9 %, respectively. Notably, AscrRNA3/AsCas12a failed to induce any observable mutant phenotypes. In terms of temperature stability and editing efficiency, LbCas12a demonstrated superior performance relative to AsCas12a. Additionally, there was no statistically significant difference in the phenotypic outcomes between the co-injection of the three crRNAs and the individual injection of each crRNA.

In summary, elevating the temperature significantly augments the editing efficiency of Cas12a, with the highest frequency of the albino phenotype observed at 29.5 ◦C. To further elucidate the temporal dy-namics of LbCas12a and AsCas12a activity, we examined the effects of varying durations of exposure to 29.5 ◦C on phenotype manifestation. Edited embryos were incubated at this elevated temperature for 1, 2, 3, 4, 5, 9, 12, and 24 h, subsequently being transferred to a standard temperature of 23.5 ◦C. Our findings demonstrate that a brief incubation period of 1 h at 29.5 ◦C suffices to achieve a substantial knockout efficiency of the tyr gene.Extension of the high-temperature incubation period did not yield atistically significant enhancement in the proportion of the albino phenotype.