**News Release**

Shedding Light on the Evolutionary Engine of Cancer
-Optimized CRISPR-Cas9 system for efficient engineering of ecDNA in cancer cell-

## Key Points

- In recent years, circular extrachromosomal DNA (ecDNA) has attracted significant attention as a key mechanism underlying cancer evolution, intratumoral heterogeneity, and therapeutic resistance. However, the functions and dynamics of ecDNA within cancer cells have remained poorly understood, in part because no established technology has been available to artificially manipulate ecDNA.
- By optimizing the guide RNA used for CRISPR-Cas9-mediated genome editing—through the use of a “safeguard sgRNA”—we have successfully established an efficient method for genome editing of ecDNA.
- We systematically demonstrated that conventional CRISPR–Cas9 induces excessive DNA cleavage of ecDNA, leading to cell death and loss of ecDNA, whereas the use of a “safeguard sgRNA” enables efficient genome editing while suppressing cytotoxicity.
- Computational simulations achieved a systematic understanding of cellular responses to DNA cleavage events occurring in multicopy ecDNA.
- The optimized ecDNA genome-editing strategy established in this study represents a foundational technology that enables functional analyses and dynamics of ecDNA, which play critical roles in cancer malignancy and drug resistance. This approach is expected to make a substantial contribution to the future development of ecDNA-targeted cancer research and therapeutic strategies.

## Summary

A research group led by Prof. Hiroshi I. Suzuki and a graduate student Yohei Sugimoto, Department of Molecular Oncology, Nagoya University Graduate School of Medicine, together with Masaki Kawamata, Assistant Professor at the Kyushu University, has successfully developed a technology that enables efficient genome editing of extrachromosomal DNA (extrachromosomal DNA; ecDNA), which is present in large numbers in cancer cells, using CRISPR–Cas9.

In recent years, ecDNA has attracted increasing attention as a key mechanism underlying cancer evolution, intratumoral heterogeneity, and therapeutic resistance. ecDNA is a unique form of circular DNA that contains genes strongly promoting cancer cell proliferation and is found in many cancer cells, often in dozens of copies within a single cell. Because ecDNA is unevenly distributed during each round of cell division, its copy number varies among cells. This heterogeneity is thought to contribute to increased malignancy, treatment resistance, and cancer recurrence. Therefore,

investigating the functions of ecDNA is essential for understanding the fundamental nature of cancers that harbor ecDNA.

To gain a detailed understanding of ecDNA function and behavior, it is crucial to modify ecDNA sequences using technologies such as CRISPR–Cas9, enabling visualization and functional analysis of ecDNA. However, many aspects of ecDNA function and dynamics within cancer cells remain unclear, in part because techniques for artificially manipulating ecDNA have not yet been established.

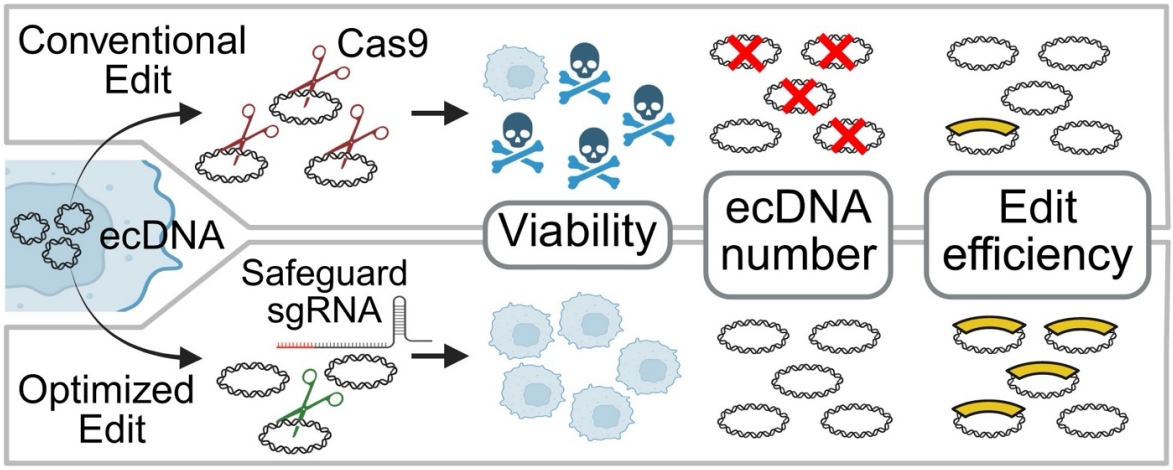
In this study, the researchers sought to develop an optimal genome-editing method for ecDNA by comparing standard CRISPR–Cas9 with a “safeguard sgRNA,” previously developed by the group, which allows fine control of CRISPR–Cas9 cleavage activity.

Interestingly, when standard CRISPR–Cas9 was applied to ecDNA, the efficiency of ecDNA editing was low, while pronounced cell death and a marked reduction in ecDNA were observed. These phenomena suggest that excessive DNA cleavage occurring simultaneously in the many copies of ecDNA within a single cell imposes severe stress on the cell. As a result, it became clear that standard CRISPR–Cas9, which is widely used across many fields, is inherently unsuitable for genome editing of ecDNA.

To overcome this limitation, the researchers suppressed cleavage activity to an appropriate level using the safeguard sgRNA. This approach enabled highly efficient genome editing of ecDNA while avoiding ecDNA loss. Furthermore, by combining experimental data with computational simulations, the team identified the degree to which DNA cleavage events in ecDNA are temporally concentrated as a critical factor determining cell survival, ecDNA maintenance, and the success of ecDNA genome editing.

The results of this study make it possible to efficiently edit ecDNA, which has previously been difficult to manipulate genetically. This advance is expected to enable more detailed investigations into how ecDNA behaves within cells and how it contributes to cancer progression and therapeutic resistance.

This research was published online in Nucleic Acids Research on January 14, 2026.



## Research Background

Cancer is a disease that arises and progresses through the accumulation of DNA abnormalities. Previous studies have revealed that genomic alterations in cancer cells are not composed solely of simple mutations in genes, but can also include abnormalities with more complex structural features. One representative example is extrachromosomal DNA (extrachromosomal DNA; ecDNA).

ecDNA originates from genomic regions that were originally located on chromosomes but exist within the cancer cell as circular DNA molecules (Fig. 1). ecDNA frequently contains genes that strongly promote cancer cell proliferation and, unlike normal chromosomes, is not evenly segregated during cell division. As a result, even within the same tumor tissue, individual cells harbor different numbers (amounts) of ecDNA, leading to intratumoral heterogeneity (Fig. 1). This property is considered to be one of the key factors enabling cancers to adapt to environmental changes, acquire drug resistance, and recur after treatment.

Despite the critical role of ecDNA in cancer evolution and malignancy, many aspects of its function and behavior remain poorly understood. One major reason for this knowledge gap is the lack of well-established technologies for directly manipulating ecDNA at the genomic level. To elucidate ecDNA function, genome editing of ecDNA is essential—for example, to modify specific sequences to visualize ecDNA dynamics within cells using fluorescent proteins. However, the utility of such analytical approaches has not been fully evaluated to date.

In recent years, advances in genome-editing technologies, typified by CRISPR–Cas9, have enabled the editing of chromosomal DNA. However, ecDNA differs fundamentally from conventional chromosomal DNA in that dozens of copies can exist within a single cell. Most previous CRISPR–Cas9 studies have been conducted under the assumption of diploid cells with two alleles, and thus there has been little systematic evaluation of the effects and cellular responses elicited when CRISPR–Cas9 is applied to ecDNA present in high copy numbers within a single cell.

Against this background, the present study aimed to identify the optimal genome-editing conditions required to manipulate ecDNA safely and efficiently.

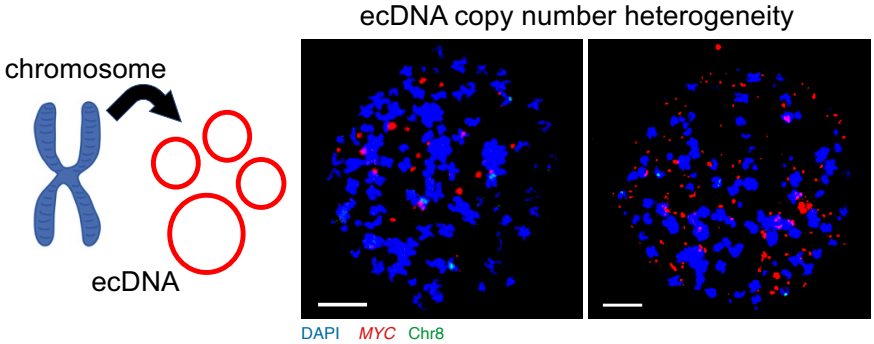



Fig. 1. ecDNA in cancer cells

**Research Results**

In this study, we analyzed the cellular responses elicited by CRISPR–Cas9–mediated genome editing targeting ecDNA present in cancer cells (Fig. 1). When standard CRISPR–Cas9 was applied to ecDNA, frequent occurrences of cell death and loss of ecDNA were observed. These results clearly indicate that the conventional CRISPR–Cas9 system, which is widely used across many fields, is inherently unsuitable for genome editing of ecDNA.

Using DNA FISH to quantify the amount of ecDNA per cell, we found that in cell populations treated with standard CRISPR–Cas9, the number of cells retaining high levels of ecDNA was markedly reduced. In contrast, analysis of cells positive for markers of cell death revealed that these cells tended to harbor high amounts of ecDNA. These findings suggest that cells containing larger quantities of ecDNA are more susceptible to the detrimental effects of standard CRISPR–Cas9 treatment and are more likely to undergo cell death.

Further microscopic observation revealed that, following treatment with standard CRISPR–Cas9, ecDNA was frequently segregated from the nucleus into micronuclei. In most cases, ecDNA was detected within these micronuclei, suggesting that after being cleaved, ecDNA is expelled from the nucleus. Together, these results indicate that CRISPR–Cas9–mediated editing of ecDNA has a profound impact on ecDNA maintenance.

In contrast, the present study demonstrated that these issues could be substantially mitigated by using a safeguard sgRNA (Fig. 2), which allows precise modulation of Cas9 cleavage activity. Under conditions in which Cas9 activity was moderately suppressed, the frequency of cell death was greatly reduced, and the proportion of cells in which genome editing could be achieved without decreasing the number of ecDNA copies per cell was increased. Moreover, when ecDNA knock-in experiment was performed under these conditions, the fraction of ecDNA molecules carrying correctly inserted genome sequences for ecDNA visualization increased, as did the proportion of cells retaining large numbers of such modified ecDNA molecules (Fig. 3). These results demonstrate that appropriate control of Cas9 activity enables highly efficient genome editing of ecDNA without reducing ecDNA copy number.

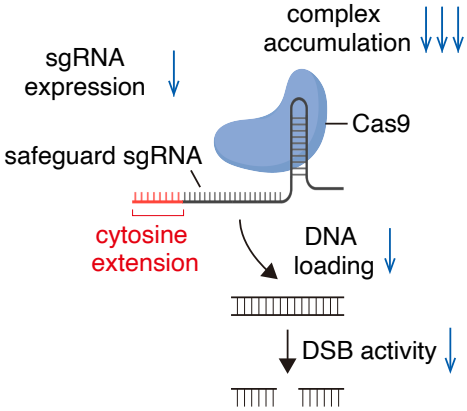



Fig. 2. Safeguard sgRNA strategy

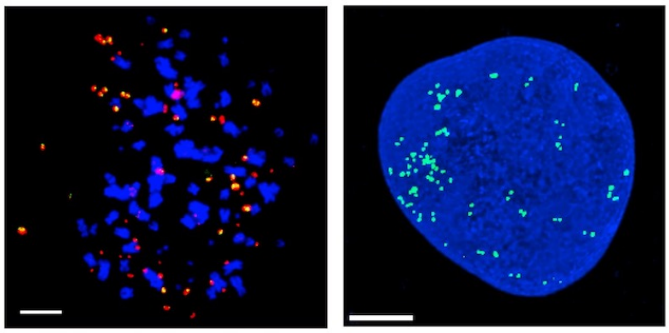


Fig. 3. Successful ecDNA knock-in (left) and ecDNA visualization (right)

In addition, this study employed computational simulations to elucidate the mechanisms underlying the experimental observations described above (Fig. 4). Specifically, we performed simulations in which DNA cleavage events in ecDNA were randomly generated along a virtual time axis at frequencies determined by Cas9 activity. The results revealed that cell survival and maintenance of ecDNA copy number depend not only on the total amount of DNA cleavage, but also critically on whether ecDNA DNA breaks are concentrated within a short time window.

When a safeguard sgRNA was used, DNA cleavage events in ecDNA were temporally dispersed, reducing the likelihood that cells would sustain lethal damage and increasing the probability that genome editing could be successfully achieved while retaining ecDNA.

Together, these findings provide a new conceptual framework for genome editing of ecDNA, demonstrating that rather than “cutting strongly,” it is essential to induce the minimum necessary level of DNA cleavage to achieve effective editing (Fig. 5).

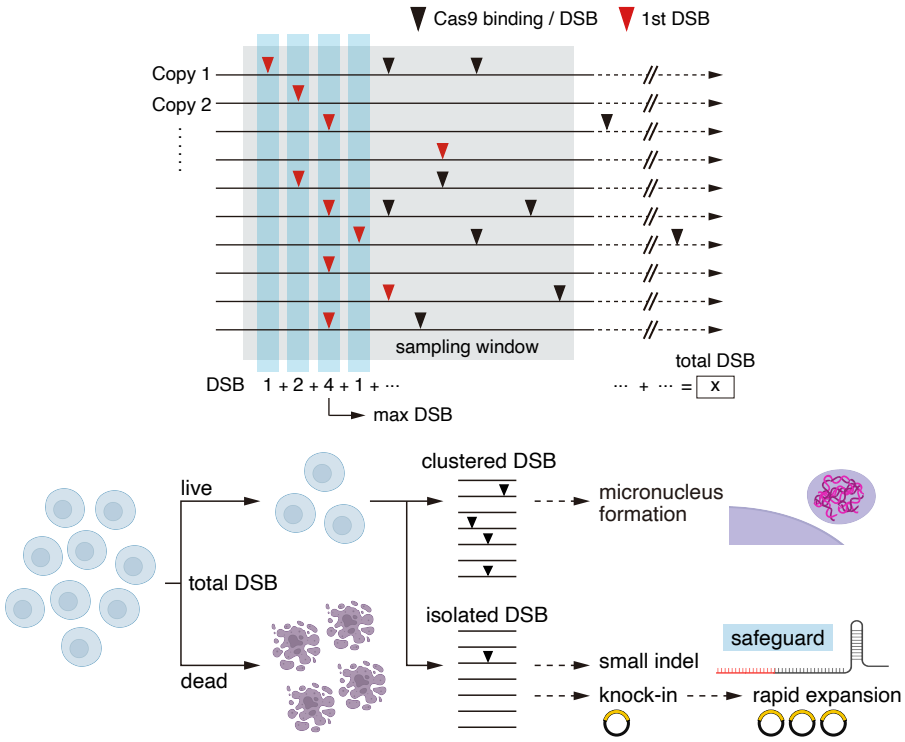



Fig. 4. Computational simulations of ecDNA cleavage events (top) and cellular consequences (bottom)

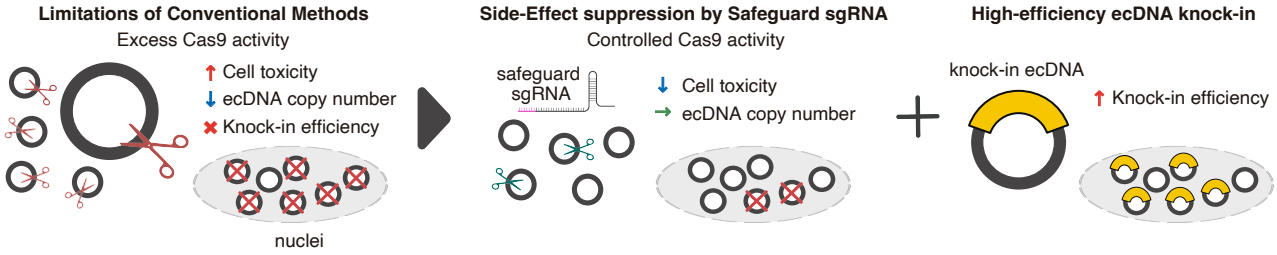



Fig. 5. Summary of the present study

## Research Summary and Future Perspective

This study has clarified the optimal conditions required for successful genome editing of ecDNA. As a result, it is now possible to efficiently edit ecDNA, and the use of this approach is expected to greatly advance functional analyses of ecDNA in future studies.

The optimized ecDNA genome-editing strategy presented in this study is anticipated to accelerate basic research focused on ecDNA and to serve as a technological foundation for opening new avenues in cancer research in the future.

## Publication

Nucleic Acids Research

Title : Optimized CRISPR-Cas9 system for efficient engineering of ecDNA in cancer cells

Authors : Yohei Sugimoto[1], Takeru Kachi[1], Yu Watanabe[1,2], Mei Kubokawa[1,3], Koichi

Ogami[1], Masaki Kawamata[4], Seiko Yoshino[1], Hiroshi I. Suzuki[1,5,6,7],
1 Division of Molecular Oncology, Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan
2 Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan
3 Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, Nagoya, Japan
4 Division of Organogenesis and Regeneration, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan.
5 Institute for Glyco-core Research (iGCORE), Nagoya University, Nagoya, Japan
6 Center for One Medicine Innovative Translational Research (COMIT), Nagoya University, Nagoya, Japan
7 Inamori Research Institute for Science (InaRIS), Kyoto, Japan

DOI：10.1093/nar/gkag005

Japanese ver.
https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Nuc_260126.pdf