ENHANCING THE FUNCTION OF THE PROOFREADER IN COLORECTAL CANCER STEM CELLS USING CRISPR TECHNIQUES

Evan Liu*

Abstract: Colorectal cancer (CRC) is one of the most prevalent malignancies worldwide. Colorectal cancer stem cells (CCSCs) play a significant role in the development of therapeutic resistance and recurrence of the cancer. These cells exhibit self-renewal and pluripotency, which makes them resistant to conventional treatments. The deficiencies in DNA proofreading—particularly the exonuclease activity of DNA polymerases—might lead to a higher probability of mutations and genomic instability. This conceptual paper proposes a novel prior prevention of colorectal cancer that combines CRISPR-Cas9 gene-editing technology with enhanced DNA proofreading capacity in CCSCs. By using CRISPR to either correct mutations in exonuclease domains or fuse high-fidelity polymerases with genome-editing tools, we can potentially reduce the probability of mutation and improve therapeutic outcomes. We discuss the background, proposed methodology, possible results, and implications, integrating insights from recent single-cell RNA sequencing studies and gene therapy trials.

Keywords: DNA Polymerases; CRISPR-Cas9; Cancer Stem Cells

* Kang Chiao International School, China.

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INTRODUCTION

A. What Are Cancer Stem Cells?

Cancer stem cells (CSCs) constitute the fraction of the tumor with stemlike properties including self-renewal, differentiation, and tumorigenicity. In colorectal cancer, these cells have been termed colorectal cancer stem cells (CCSCs) and they play critical roles in tumor initiation, progression, metastasis, and recurrence as well. The species marker of colorectal cancer stem cells has been confirmed (e.g., CD133, LGR5). [https://pubmed.ncbi.nlm.nih.gov/38156967/]. In fact, CCSCs should be targeted because conventional therapies that fail to eliminate them result in recurrences.

B. DNA Proofreading

In the course of Cell division and DNA replication, DNA proofreading normally occurs. The main utilization of DNA proofreading to sustain the fact that information regarding the sequence of nucleotides in DNA is accurate. As nucleotides are added to be copied, new strand-forming enzymes like DNA polymerases continue adding them. Occasionally, errors do take place, and the wrong nucleotides get incorporated. So proofreading works somewhat like a scanner spotting the error and taking out the wrong piece before moving on. This greatly reduces errors that could give rise to harmful mutations. When proofreading is defective, because of a mutation in the proofreading enzyme, DNA errors can accumulate.

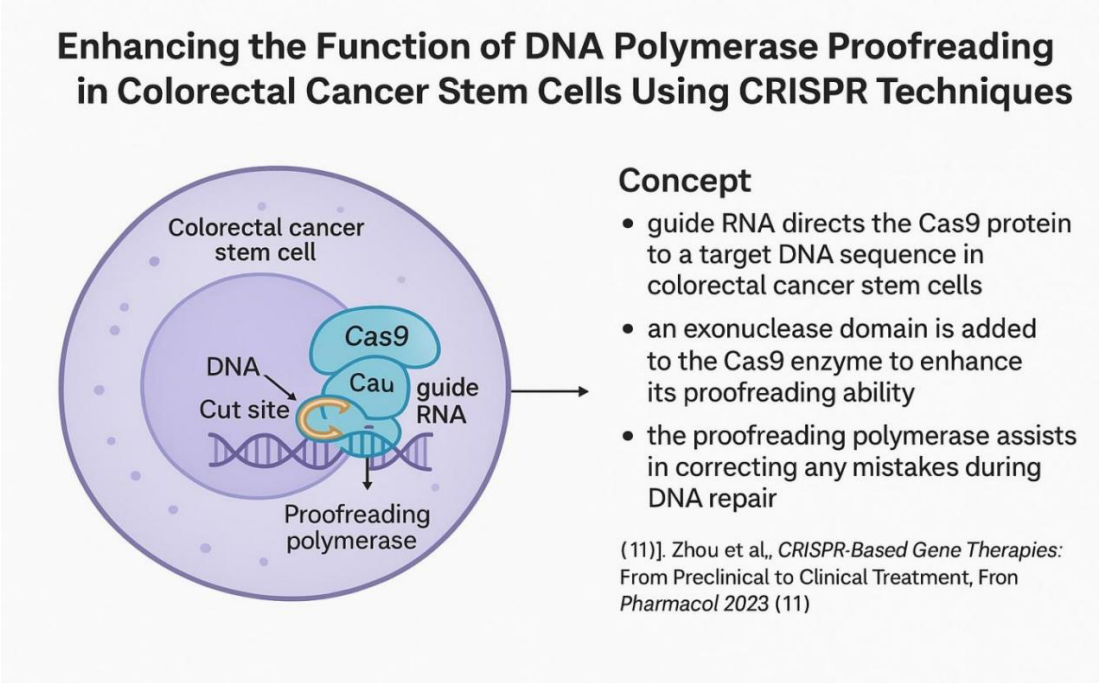
Over time, this may lead to genomic instability, cancer. Healthy proofreading systems are, therefore, absolutely critical to avoid cancer and maintain cellular stability.

C. CRISPR-Cas9

Cas9 is a kind of scissors at the molecular level that makes a cut in DNA at any specific place. It is guided by RNA to find the target gene and make a precise cut. Scientists combine this with other components to study diseases or develop therapies, such as cancer therapy by editing genomes through driving out, fixing, or adding DNA sequences. Cas9 is an accurate programming tool for genome editing. In cancer treatment, CRISPR was applied to delete oncogenes and rewire immune responses. CRISPR-based gene therapies have already made their way into clinical trials. [https://pmc.ncbi.nlm.nih.gov/articles/PMC11119143/].

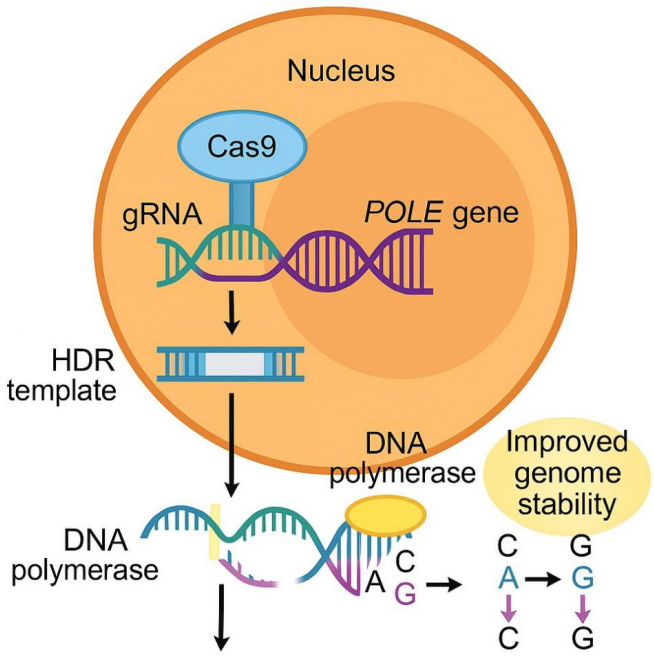
D. Enhancing Proofreader via CRISPR-Cas9

This paper suggests the application of CRISPR technology to enhance proofreading in colorectal cancer stem cells (CCSCs). These are mainly three ideas that we put forward: First, Repairing mutations in the exonuclease domain of DNA polymerases. These are enzymes responsible for correcting errors made during DNA replication. Second, Utilizing CRISPR activation (CRISPRa) to upregulate antimutator polymerases which are special polymerase variants with inherently lower error rates. Third, Engineering a Cas9 protein fused to proofreading domains so that the gene-editing tool can correct its own off-target mistakes during DNA editing. These strategies would likely increase the genomic stability of CCSCs, sensitize these cells to therapeutic interventions, and preclude them from acquiring further mutations leading to therapy resistance.



This image illustrates a proposed method to enhance DNA proofreading in colorectal cancer stem cells (CCSCs) using CRISPR-Cas9 technology. In this concept, the RNA will be directly guided to the Cas-9 protein to a specific location within the POLE gene, which is a key for proofreading DNA polymerase. Cas9 introduces a cut at the spectacular site, which allows exonuclease domain to insert or correct through homology-directed repair. To further improve the accuracy of the system, a proofreading polymerase is involved to fix any DNA mismatches during or after the editing process. This combined strategy is targeted to reduce mutation rates in CCSCs, improve genome stability, and potentially make cancer cells less resistant to treatment. The approach represents an innovative way to prove that CRISPR is not only for gene correction but also for enhancing natural DNA repair mechanisms in cancer therapy (Zhou et al., 2023).

I. Detailed Methodology and Concept Diagram



This figure illustrates the conceptual framework for enhancing DNA proofreading in colorectal cancer stem cells (CSCs) using a CRISPR-Cas9 system. The CRISPR complex is shown targeting the *POLE* gene within the nucleus of colorectal CSC. Through homology-directed repair (HDR), the exonuclease domain is engineered to enhance proofreading activity, reducing mutation rates during replication. DNA polymerase is shown actively removing mismatches. The image includes labels for Cas9, gRNA, HDR template, POLE gene, DNA polymerase, and a highlighted area showing improved genome stability post-editing.

A. Concept Overview

This study is based on a hypothesis that we will encourage DNA polymerase proofreading in colorectal cancer stem cells (CCSCs) to suppress the mutation rate and genome stability and thereby make cancer more drug-sensitive. This strategy is based on newly sophisticated CRISPR-Cas9 gene editing technology driven by recent RNA-sequence-based analysis and further develops their previous work toward adaptation of CRISPR-based gene therapy to initial testing in clinical settings (PMC11119143).

B. Strategy Summary

We propose a three-pronged strategy using CRISPR to enhance proofreading fidelity within CCSCs:

1. **Targeting the colorectal gene via repairing or enhancing the exonuclease domain** of faulty DNA polymerase genes (e.g., POLE) using CRISPR-Cas9 with homology-directed repair (HDR).

2. **Activating transcription** of antimutator DNA polymerase variants using CRISPR activation (**CRISPRa**) to suppress mutagenic replication behavior.

3. **Engineering Cas9 fusion proteins** linked to proofreading domains, providing real-time error correction during editing events.

This integrative approach seeks not only to fix existing proofreading deficits but to build a **self-correcting gene editing platform** tailored to the molecular needs of CCSCs.

II. DISCUSSION

The major issue is how to target the therapies to only the colorectal parts of the gene. Although the "marker"-LGR5, CD133- for colorectal cancer stem cells has been discovered, these are single markers, which present in normal intestinal stem cells. Thus, when the therapy is exploited, it will annihilate every cell that has the marker, making the process difficult to target only colorectal cancer stem cells. Moreover, the expression of cells can vary between tumor locations and patients, resulting in an inaccurate process.

In my opinion, to deal with the issue I have mentioned, further development should be focused on the specific marker for Colorectal Cancer Stem Cells that only CCSCs contain, so the therapy can be injected effectively.

The accuracy of DNA replication is essential for the maintenance of cellular identity and tissue function. In colorectal cancer stem cells (CCSCs), which are reportedly responsible for the reprogramming of tumors, fidelity is often relaxed through mutations in proofreading enzymes like POLE and POLD1. These defects are known to be associated with increased mutation burdens, genomic instability, and poor treatment response. Treatment strategies currently in use do not target this genetic instability in CCSCs, which also plays a role in permitting the survival and evolution of cancer cell populations that can adapt to various conditions.

To overcome this obstacle, here we develop a CRISPR-based approach to augment DNA proofreading in CCSCs. Specifically, there are three approaches we propose: (1) editing the pathogenic mutations within exonuclease domains of polymerase genes using CRISPR-Cas9 and homology-directed repair (HDR), (2) activating expression of antimutator polymerase variants via CRISPRa to reduce replication errors, and (3) engineering a Cas9–exonuclease fusion protein to facilitate error-correction gene edits. These supplements have been developed to rebalance CCSC genomes, decrease mutation-induced plasticity and increase treatment fidelity.

However, several limitations must be acknowledged. Understanding how proofreading enzymes distinguish between newly synthesized DNA strands and parental DNA strands remains a fundamental concern. Healthy cells utilize methylation and structural indicators to distinguish DNA strands which enables precise proofreading. CCSCs present extensive mutations on both DNA strands which can interrupt the recognition process. Enhanced proofreading mechanisms can inadvertently sustain faulty sequences when relying on templates that contain mutation-induced errors. This raises a significant challenge:

CRISPR-induced proofreading edits in hypermutated CCSCs may have the potential to strengthen genomic instability instead of fixing it.

The precise moment when the intervention occurs becomes essential. Maximizing correction fidelity requires administering CRISPR-enhanced proofreading systems early in tumor development before widespread mutations accumulate. The current situation indicates the need for advanced research to find early CSC populations and develop delivery mechanisms that can accurately target them.

Future investigation should address several key questions:

(1) How can we make sure the CRISPR system knows which DNA strand to fix when both strands have the same marker, but only one has cancer?

(2) Can we use lab-made DNA templates to help CRISPR fix the right parts in cells that already have a lot of mutations?

(3) Are there special genes or markers we can use to find cancer stem cells early, so we know exactly which cells to target with treatment?

(4) Does the environment around the tumor affect how well the improved proofreading system works, and could it make the system less or more effective?

The combination of single-cell RNA sequencing (scRNA-seq) improvements with new stemness signatures like SCS_sig can facilitate detailed mapping of CCSC populations throughout tumor development stages and patient groups. This method of measuring stemness as a spectrum rather than a binary trait enables the identification of patients who will gain the most advantage from CRISPR-based proofreading therapy.

The research establishes a conceptual framework that utilizes precision gene-editing techniques to stabilize the CCSC genome while minimizing the incidence of mutation-induced resistance. This approach shows promise yet requires additional experimental validation to understand DNA repair processes within highly mutated cancer genomes. The integration of computational analysis with experimental findings could produce innovative treatments that redirect colorectal cancer development towards manageable therapy outcomes.

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