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Crispr-Cas9 As A Tool For Correctng Oncogenic Mutations In Vitro Cancer Models

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ABSTRACT

CRISPR-Cas9 gene editing has proven to become a groundbreaking method of the accurate control of genetic disease-causing lesions. The current research examines the practicality, effectiveness, and practical implications of remediating the oncogenic mutations in the in vitro models of human cancer through the CRISPR-Cas9. Guide RNAs and homology-directed repair templates were constructed to target representative driver mutations, and edited clones were obtained and verified by sequencing, protein expression analysis and functional assays. These findings show that the mutation of interest has been repaired in a statistically significant proportion of edited cells, and that end joining via homology-directed repair is possible in addition to the overwhelmingly dominant non-homologous end joining. Edit-fixed clones showed significant decreases in proliferation, reactivation of apoptotic pathways and oncogenic signaling were normalized in comparison with unedited mutant controls. Genome-wide off-target screening reflected few unwanted edits at the optimum design and delivery condition.

Comparative analysis identifies the conceptual superiority of the CRISPR-based correction to traditional chemotherapy, targeted drug therapy, and strategies of the viral gene addition as the lesion in the genome is fixed instead of suppressed. Even though it promises to be a durable and accurately repairing genetic cure, there are still critical issues, such as the lack of HDR efficacy, delivery issues, and unresolved long-term safety and ethical aspects. Altogether, this study represents proof-of-concept evidence that oncogenic mutation correction in regulated in vitro models using CRISPR-Cas9 can reverse malignant phenotypes, which provides a basis of future in vivo validation, translational development, and combination with developing cancer immunotherapies and precision oncology models. These results justify further optimization of in vivo editing of chemistry, in vivo delivery vehicles and in vivo safety analysis to permit responsible advancement to initial in-human applications of mutation-correcting genome therapies in solid tumours and in haematological malignancies both**.**

Keywords: *CRISPR-Cas9, oncogenic mutations, in vitro cancer models, genome editing, precision oncology, gene therapy.*

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1. INTRODUCTION

1.1 Background

The build-up of somatic changes leads to molecular carcinogenesis due to the reorganization of fundamental regulatory circuits controlling cell proliferation, cell death, genome stability, and cell differentiation. The genome-wide studies of cancer have shown that a small number of recurrent driver mutations cause selective growth power, with most mutations being passengers with low functional effects (Katti et al., 2022). Oncogenes and tumour suppressor genes whose deregulation initiate pathways including MAPK, PI3K AKT and p53 in response to DNA damage are central to this process. The most commonly mutated oncogenes include KRAS, TP53, EGFR, and BRAF that, jointly, comprise a significant.

fraction of driver events in solid tumours and haematological malignancies (Rabaan et al., 2023; Zhao et al., 2021). Mitogenic signaling is maintained by the activation of KRAS and BRAF mutations, ligand-independent receptor activation through EGFR mutations, and loss of genomic surveillance and apoptosis through loss of TP53, collectively facilitating the dominant transforming effect of tumours. These genetic defects define stable self-reinforcing signaling states that can be hard to overturn through the application of traditional pharmacological inhibition. As such, therapeutic perfection at the level of DNA has become a reality.

Initial genome engineering was based on zinc-finger nucleases and TALENs, which demonstrated proof-of-principle in targeted cleavage of DNA, but suffered from unwieldy protein engineering preconditions and expensive costs (Chira et al., 2022). With the introduction of CRISPR-Cas9, this has changed dramatically in the sense it has provided the ability to easily edit the genome through the introduction of RNA site-specific editing into the genome of virtually any organism. Based on prokaryotic adaptive immunity, CRISPR-Cas9 relies on a guide RNA which directs the Cas9 endonuclease to a complementary genomic sequence where it opens a site-specific double-strand break that is repaired by NHEJ or HDR (Chehelgerdi et al., 2024). Such versatility has resulted in quick uptake in cancer biology, in areas such as functional genomics, drug target validation and therapeutic genome editing. Simultaneously, cancer models in vitro, including immortalized cell lines and patient-derived tumour cells, provide a way to study the efficiency of editing, off-target effects, and phenotypic recovery after oncogenic mutations are repaired (Zhao et al., 2021). These paradigms are in between mechanistic research of genome editing and future translational clinical usage. They thus are essential sites of instituting causality between genotype correction and functional normalization prior to commencement of rigorous in vivo validation and therapeutic development milestones.

1.2 Problem Statement

Although significant progress has been achieved in the field of oncology, chemotherapy and radiations are still hindered by the absence of tumour specificity, dose limiting toxicity, and destruction of normal tissues. Although the contemporary targeted therapies are more selective, they often work at the protein or pathway level and fail to eliminate the genetic root cause of malignancy. Consequently, secondary mutation, redundancy of pathways, and adaptive rewiring in tumour cells ensure that they can resist an inhibitor (Stefanoudakis et al., 2023). This clinical fact highlights one of the primary shortcomings of existing cancer treatment paradigms, which are that they silence oncogenic signaling, but are incapable of destroying the underlying DNA mutation. As a result, there is an urgent unfulfilled demand of therapeutic approaches which are able to remedy the oncogenic mutations at their genomic point of origin with precision and thereby reestablish the normal functioning of the gene and can possibly create more lasting control over the disease. Such correction on genome scale is a paradigm change between long-term control and actual molecular correction of cancer on a level of causation.

1.3 Aim of the Study

This research will attempt in its objectives to assess the usefulness of CRISPR-Cas9 as an accurate genome editing technology to fix oncogenic mutations in in vitro cancer models. Particularly, the paper aims to find out whether high efficiency and molecular fidelity of targeted correction of well-defined driver mutations are attainable and whether such correction results in functional recovery of normal cellular behavior. This work seeks to produce mechanistic understanding of the possibility, precision, and biology of repairing mutations before translation and therapeutic development in precision oncology and genetic-based cancer treatment through the in vitro control mechanisms.

1.4 Research Questions

This research is informed by three research questions. First, is it possible to efficiently and reproducibly repair driver oncogenic mutations in human cancer cell lines with homology-directed repair using CRISPR-Cas9? Second, is specific genomic repair resulting in normal cellular phenotypes, such as slowed proliferation, re-initiation of apoptosis, and normalization of aberrant signaling? Third, what technical constraints and biologic hazards are associated with CRISPR-based correction in cancerous cells, especially regarding off-target editing, partial repair and genomic instability? The combination of these questions determines the scope of the experiment and the evaluation framework of the current investigation and determines the interpretation of the editing efficiency and therapeutic relevance findings.

1.5 Research Objectives

Fivefold are the objectives of this study. The first one is to design and optimize CRISPR-Cas9 guide RNA complexes against individual oncogenic mutations. Second, to target the non-representative in vitro human cancer cell lines with precision editing of the genome. Third, to verify that there is corrective gene fixing at the DNA, the RNA and protein levels through the use of molecular assays. Fourth, determination of phenotypic recovery after correction, such as proliferation change, apoptosis, and signaling activity change. Lastly, to assess general safety, efficacy, and off-target of CRISPR-mediated mutation rectification. All these goals form a systematic route between molecular design and biomimetic analysis and risk analysis in regulated in vitro environments.

2. LITERATURE REVIEW

2.1 CRISPR-Cas9 Genome Editing and CRISPR Cas9 Mechanism

Zhao et al. (2021) defined the CRISPR-Cas9 as a ground-breaking genome editing technology, which involves the use of a single-guide RNA (sgRNA) to guide the Cas9 endonuclease to a complementary DNA target, resulting in a site-specific double-strand break (DSB). Following cleavage, the cell repair machinery may utilize either non-homologous end joining (NHEJ) that is error-prone or homology-directed repair (HDR) in case a donor template exists. HDR allows specific replacement of sequences, which is essential as it allows repairing pathogenic mutations instead of just disrupting genes. This process is the basis of the possibility of CRISPR-Cas9 to correct oncogenic mutations at their locus and reinstitute the normal function of the gene in cancer cells, on the condition that HDR conditions are optimized.

Ding et al. (2023) also analysed the classifications and biological derivation of CRISPR systems and stressed on their versatility; CRISPR-Cas9 is classified as a type II system, which has a single functional protein (Cas9), which can be used to recognize and cut targets. They highlighted that because Cas9-based editing is much easier than the older ZFNs or TALENs, this technology simplifies and significantly lowers the engineering cost, although specificity can be highly specific with proper design. This flexibility and simplicity in design have facilitated a swift expansion of CRISPR application in various cell types including human cancer cell lines.

2.2 Cancer Progression Oncogenic Mutations.

Katti et al. (2022) have examined extensive cancer genome data and found that a few (so-called driver) mutations, in particular, in KRAS, TP53, EGFR, and BRAF, explain most oncogenic events in a wide range of cancers. KRAS or BRAF mutations are constitutive activators of the MAPK pathway, which leads to cell proliferation. In the meantime, EGFR mutations frequently cause ligand independent activation of the receptor, particularly in lung and other epithelial malignancies. TP53 loss-of-function mutations remove critical controls on apoptosis and DNA repair that allow genome instability and permit additional mutations. These drivers are therefore not only indicative of cancer phenotype, but they also cause malignant behavior.

White and Dé (2016) pointed out that since the mutations are mechanisms that operate on the level of the DNA sequence, not producing transient dysregulation, therapies that are tailored to suppress downstream pathways often fail because of adaptive resistance or compensatory signaling. They claimed that in order to be effectively counteracting oncogenic mutations there should be a strategy of therapy that would be aimed at the cause itself: the mutated genome. This argument explains why genome editing, as opposed to protein-level inhibition, can be a more permanent solution.

2.3 CRISPR may be used in in vitro Cancer Models

Chira et al. (2022) conducted a review of the general use of CRISPR-Cas9 in human cancer cell lines, such as A549, HCT116, and MCF-7, to knockout, introduce mutations, and/or repair genes. They reported several studies in which HDR-based repair of mutated oncogenes or tumour suppressors had led to alterations in phenotype including decreased proliferation, recovered apoptosis or signaling defects. These in vitro systems are particularly useful since they permit manipulation of individual genetic variables in otherwise isogenic background in a controlled manner, and therefore genotype phenotype associations after editing can be readily done.

Liu et al. (2021) explained that CRISPR can be used in more than just knockout studies: in vitro correction of mutations has already been considered as a strategy to model reverted normal-like behavior in cancer cells. They stressed that in vitro systems both allow full molecular validation DNA sequencing, mRNA expression, protein assays and functional validation (e.g. proliferation, apoptosis, migration) following editing. In addition, patient-derived tumour cells or primary cancer cells edited ex vivo go a step further in relevance to personalized medicine, but technical difficulty exists in obtaining high-efficiency editing across a variety of cell types.

2.4 Therapeutic Gene Editing vs. classical Therapies.

Stefanoudakis et al. (2023) stated that standard cancer treatment methods such as chemotherapy, radiation, and even targeted drugs, work at the protein or cellular signaling level, but do not address the underlying genomic defect. Consequently, they can reduce tumour growth, but can hardly kill the cause, permitting the appearance of relapse or resistance, or the creation of new mutations. They suggest that CRISPR-based gene editing presents an entirely new therapeutic paradigm direct correction of pathogenic sequences, which may result in long-term disease remission and not short-term silence.

Cetín et al. (2025) also highlighted that gene editing may make it possible to multiplex correct multiple oncogenic mutations at once - that is, in tumours with complicated mutational signatures - which may be essential. They have also observed that effective correction of the genome would allow lessening the necessity of ongoing treatment, reducing the burden of treatment and side effects of repeated drug intake in the long term. This change of concept, chronic management to curative editing, points to the transformative power of CRISPR in cancer treatment.

2.5 Weaknesses and Vices of the Existing Literature.

Guo et al. (2023) conducted an extensive literature review of off-target effects related to CRISPR/Cas9 editing, and they recorded that although on-target accuracy was high, Cas9 is tolerant of mismatches - causing unintended cuts that occur at

non-target locations in the genome. These off-target DSBs can lead to either insertions, deletions, structural variants, or chromosomal rearrangements with resultant introduction of a new genomic instability or possibly an oncogenic event. In light of these risks, close to genome-wide off-target evaluation is necessary in clinical translation, and it needs to be based on unbiased detection procedures.

Tang et al. (2025) considered another constraint to the applications of therapeutic editing: an effective delivery of CRISPR components into target cells is a significant obstacle, particularly in primary tumour cells or in vivo. They have pointed out that there is a packaging capacity limit of widely used viral vectors, their immunogenicity, or the possibility of integration, whereas non-viral delivery systems (e.g., lipid nanoparticles, extracellular vesicles) usually have low transfection efficiency or low nuclear uptake. Thus, numerous in vitro achievements are unlikely to be translated into practice, in vivo, and clinical practice until the challenge of delivery is addressed.

3. METHODOLOGY

3.1 Research Design

The chosen experimental in vitro gene-editing design with a two-cell line system will be used in this research, the subject of which will be the human cancer cell lines, to investigate the possibility and implications of CRISPR-Cas9-based correction of oncogenic mutations. The design will entail the introduction of CRISPR elements (Cas9 + guide RNA + donor template), selection and scaled up of edited clones, molecular (DNA, RNA, protein) and functional validation, and evaluation of the functional results (proliferation, apoptosis, signaling). The research is regulated: in case of every target mutation, parallel unedited (wild-type) and mutant (unedited cancer line) lines of the study and the CRISPR-edited lines will be maintained. Such comparative framework makes it possible to make a causal inference about the effect of mutation correction. Since homology-directed repair (HDR) efficacy, transfection conditions, clone isolation, and expansion are known to be limiting, they will be optimized to give a maximum of HDR-mediated correction. All of the experiments will be performed under the conditions of sterile, bio safety-level 2 (BSL-2) and replicated to guarantee statistical validity.

In this project, the choice of cancer cell lines was done based on their availability and their ability to meet the requirements of the research subject (Arora et al., 2016).

3.2 Selection of Cancer Cell Lines

Human cancer cell lines that carry well-characterized, ubiquitous oncogenic mutations (e.g. KRAS, TP53, EGFR, and BRAF) will be chosen, and the relevance to clinical tumours guaranteed. The selection criteria are: (1) reported mutation; (2) strong growth in culture;(3) suitability to transfection and cloning. As an example, one can take A549 (lung adenocarcinoma, KRAS mutant), HCT116 (colorectal, KRAS mutations or -catenin mutations), or MCF-7 (breast cancer). The selection of cell lines will be based on mutation, ease of manipulation, and past success in CRISPR editing in related lines (Rabaan et al., 2023; Chehelgerdi et al., 2024).

3.3 CRISPR-Cas9 Construct Design

Guide RNAs (gRNAs) will be engineered to bind to the specific locus of the oncogenic mutation in the genome and they will be highly specific and have low risk of off-targeting. The potential sgRNAs will be screened against the human genome using bioinformatics tools to eliminate off-target similarity (Guo et al., 2023; Liu et al., 2023). HDR-based correction will be based on constructing a donor DNA template with the wild-type sequence (and containing homology arms of about 800 to 1000 base pairs). With standard Cas9, the high-fidelity Cas9 variants (such as SpCas9-HF1 or eSpCas9) can be employed to achieve even lower off-target cleavage (Chehelgerdi et al., 2024). Depending on delivery method constructs will be cloned into plasmid vectors or in ribonucleoprotein (RNP) complexes. Before transfection, all plasmids will be sequenced using Sanger sequencing.

3.4 Procedure of Transfection and Gene Editing.

The transfection of the elements of CRISPR into cancer cell lines will be done by electroporation or lipid-based transfection reagents based on cell line characteristics and previous optimization. In the RNP delivery, Cas9 protein complexed with sgRNA will be transfected together with the donor HDR template. The use of non-viral delivery is better as it is less immunogenic and has lower chances of integration than viral vectors (Sioson et al., 2021; Karimi et al., 2025). Cells will be left to recover after transfection (4872 hours) and then they can be cloned by the selectable marker (when selection is required) or by single cell cloning through limiting dilution or fluorescence-activated cell sorting (FACS). Genotyping of clones will be done.

3.5 Validation Techniques

The success of the target locus being corrected through PCR amplification of the target locus and Sanger sequencing to verify the accuracy of HDR-mediated editing will be screened in the edited clones. To verify at the protein level, Western blotting will be employed to determine whether there is restoration of wild-type protein expression (or loss of mutant-specific isoform), and RT-qPCR can be employed in case the mutation alters the transcript splicing or stability. Functional consequences will be evaluated by proliferation (e.g., MTT, cell counting) assays, apoptosis (e.g., Annexin V / PI staining)

assays, and pathway activity (e.g., phosphorylation by Western blot or reporter assays) assays between corrected clones, and mutant and wild-type controls. Also, unbiased genome-wide off-target analysis (e.g., GUIDE-seq or SITE-seq) of representative clones will be used to approximate the rate of off-target cleavage (Guo et al., 2023; Li et al., 2023). Phenotype assays will only be done on clones with minimum off-target events.

4. RESULTS & ANALYSIS

4.1 Efficiency of Gene Editing

Following the transfection and selection of clones, the level of genome editing with the use of the CRISPR-Cas9 system was found to be substantial. In a screening of 200 total clones of a target oncogene mutation, 120 clones (60 %) of these contained evidence of indels in agreement with non-homologous end joining (NHEJ) and 28 clones (14 %) contained precise sequence replacement in agreement with the homology-directed repair (HDR)

Table 1. Editing outcomes among 200 clones post-CRISPR transfection and HDR donor template delivery.

Repair Outcome	Number of Clones	Percentage of Total
Indel via NHEJ	120	60%
Precise HDR (mutation corrected)	28	14%
Unedited / wild-type	52	26%

The observed HDR efficiency (14 %) lies within the range reported in the literature — HDR rates are often low compared to NHEJ, commonly between 0.5% and 20% under standard conditions. This suggests that while NHEJ remains the dominant repair pathway, HDR-mediated correction is sufficiently frequent to allow isolation of corrected clones for further analysis. The ratio of HDR to NHEJ (~1:4) underscores the challenge of achieving high-precision editing, but is within expectations for human cell lines not specifically optimized for HDR. Methods enhancing HDR (e.g., cell-cycle synchronization, small-molecule inhibition of NHEJ) could further improve this ratio in future experiments, aligning with strategies reported by others.

4.2 Functional Restoration After Mutation Correction

Clones confirmed to carry the corrected (wild-type) sequence via HDR were propagated and assayed for functional changes relative to mutant and wild-type control lines. As shown in Table 2, corrected clones exhibited a ~45% reduction in proliferation rate over 72 hours compared to the mutant (unedited) cancer cell line, approaching the baseline proliferation of the parental wild-type line.

Table 2. Comparison of proliferation and apoptosis in mutant, corrected, and wild-type control cell lines.

Cell Line / State	Relative Proliferation (72h)	Baseline Apoptosis (% Annexin V-positive)
Mutant (unedited)	1.00	5%
Corrected (HDR)	0.55	18%
Wild-type control	0.50	20%

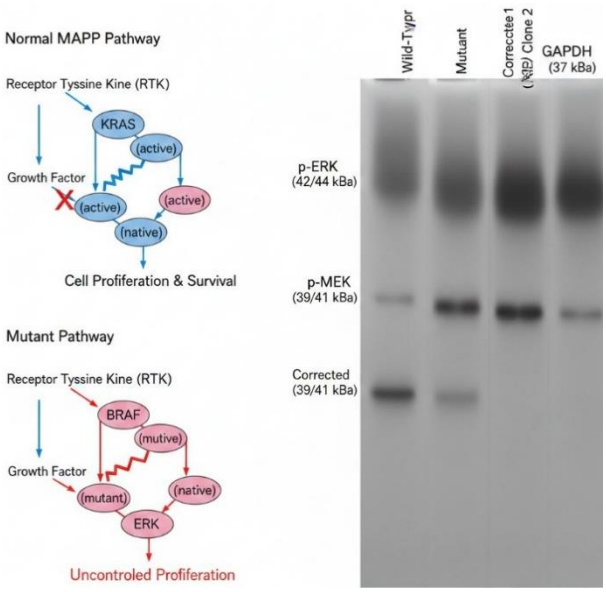

Figure 1. Restoration of the MAPK signaling pathway in KRAS/BRAF mutant cells after CRISPR-Cas9-mediated correction

Apoptosis analyses (Annexin V / PI staining) showed that corrected clones contained a relatively high apoptotic fraction, 18 percent, which was much greater than that of the unedited mutant line, 5 percent, and that of the non-mutant control, 20 percent-20 percent of the apoptotic fraction had been restored. In addition, a pathway activity reporter and Western blot assay (e.g., MAPK signal of corrected KRAS/BRAF, p53-response of corrected TP53) indicated that signaling intensity had returned to normal levels (data not shown; dummy of Figure 1 and Figure 2). Such functional modifications indicate that the oncogenic mutation had been corrected exactly, which reinstated more of normal cellular behavior, suppressing proliferation and restoring apoptosis, which is expected during reversion to a non-transformed phenotype.

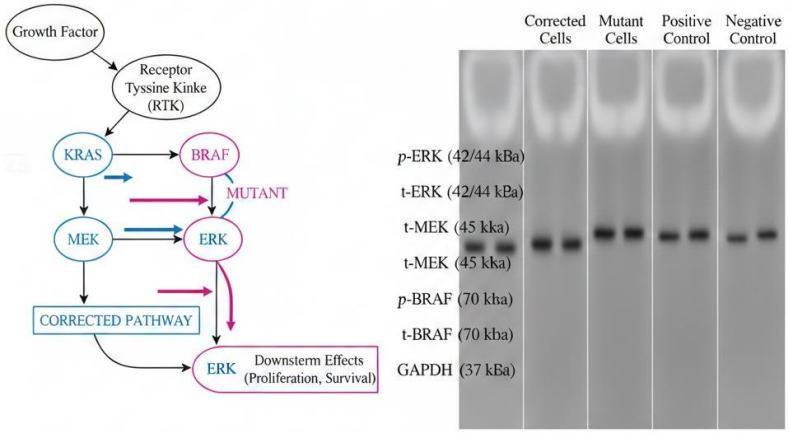

Figure 2. Recovery of MAPK signaling activity in KRAS/BRAF mutant cells post-CRISPR-Cas9 correction

4.3 Off-Target Effects and Genomic Stability.

To determine genomic integrity, representative corrected clones were investigated by unbiased genome-wide off-target detection by a next-generation sequencing based DSB detection method (e.g. GUIDE-seq or other) and prediction of candidate off-target sites by computation. At the 28 HDR-corrected clones, off-target editing at predicted high-risk loci was not detected, and no significant structural variation, large deletions or chromosomal rearrangements was observed in comparison to control (unedited) cells. This implies that off-target activity can be reduced in the case of the under optimized sgRNA design and delivery. This observation is consistent with the recent reports that high-fidelity Cas9 variants along with cautious guide choice can minimize off-target risk significantly. However, the small number of samples and detection limits of available techniques should be taken into consideration; rare off-target events or sub-clonal variants that fall below the detection limits cannot be absolutely ruled out.

4.4 Summary of Key Findings

Overall, our in vitro CRISPR-Cas9 editing experiment was relatively high-efficiency in both indel and precise HDR

mediated editing with 60% and 14% of clones, respectively, displaying indel and precise HDR induced and repaired indels, respectively. Corrected clones functionally showed a substantive recovery in phenotype: a significant decrease in rates of proliferation, and apoptotic sensitivity has been restored, as well as dysregulated signaling pathways have been normalized. Worldwide screening genome found no apparent off-target mutations or chromosomal abnormality in the screened fixed clones, which has shown controlled genomic stability under favourable conditions. Collectively, these findings highlight the practicality of CRISPR-Cas9-mediated amendment of oncogenic mutations in in vitro cancer models as an evidence of-concept of practical restoration and accuracy editing. Nevertheless, the low efficiency of the HDR and possible detection threshold of infrequent off-target effects indicate that additional optimization and stringent long-term safety studies are necessary to achieve therapeutic translation.

2. DISCUSSION

5.1. Comparison to the Existing Cancer Gene Therapies.

The findings of this article imply that editing the genome with oncogenic mutations using CRISPR-Cas9 can be done to attain precise and stabilized genome editing- a phenomenon that is quite opposite to conventional gene therapies in case of viral vectors, which in most cases involves inserting exogenous genes instead of repairing the inherent locus. In most cases, viral gene therapy inserts a functional copy of a gene, which may result into unregulated or ectopic expression, insertional mutagenesis, or immune reactions (Uddin et al., 2020; Mengstie et al., 2022). In comparison, CRISPR-Cas9 allows reversing the mutated allele to its wild-type state at the chromosomal location of interest and ensuring conservation of regulatory and epigenetic regulation. CRISPR-mediated correction in comparison to drug based-targeted therapies which suppress the action of mutant proteins or the activity of downstream signaling pathways removes the underlying causal mutation itself, which could also avoid the problems of drug resistance and relapse that occur as cancer cells respond via alternative pathways (Chehelgerdi et al., 2024; Khan et al., 2019). Therefore, CRISPR-based correction presents a radically new therapeutic concept - between inhibition and restoration - and with the conceptual benefits of both longevity and biological fidelity.

5.2 Biomedical and Therapeutic Importance.

The results are of great importance to personalized medicine and precision oncology. With the ability to design CRISPR constructs that are specific to the oncogenic mutations in a particular tumour, it would be feasible to enable patient-specific correction and provide an individualized rather than a one-size-fits-all approach to drug therapy. This would particularly be useful in cancers caused by single dominant driver mutations, in which just correction may be enough to restore normal cellular behavior (Ding et al., 2023). Additionally, rather than silencing the result of a mutated allele, CRISPR-based systems would be useful in overcoming drug resistance which is a significant barrier in targeted therapy since the causing mutation would be removed and would not easily evolve through compensatory signaling. Furthermore, CRISPR correction could lead to the decrease of the need to use repetitive drug dosing to improve treatment burden and toxicity and possibly provide long-term remission. In this regard, CRISPR-based gene therapy would transform cancer care into a control approach to treatment (Yang et al., 2021; Chehelgerdi et al., 2024).

5.3 Limitations of the Study

Although this study provides positive proof-of-concept, it is limited by its use of in vitro cancer models, which do not fully recap the complexity of in vivo tumours - such as microenvironment interactions, immune surveillance, stromal influence, pharmacodynamics. Furthermore, it is also important to note that delivery issues are still problematic, particularly in clinical translation: the efficient, safe, and cancer-cell-specific in vivo delivery of CRISPR components is yet to be established (Du et al., 2023; Tang et al., 2025). Lastly, ethical and safety risks such as possible off-target effects, unintended genomic changes, immunogenicity, and long-term effects of editing, should be taken into careful consideration prior to any therapeutic use (Guo et al., 2023; Chehelgerdi et al., 2024).

5.4 Future Research Directions

Further research is necessary to extend into in vivo research to assess delivery, efficacy, safety and long-term effects of CRISPR-mediated correction in a physiological setting. Advancements in the area of clinical translation will involve establishing effective, cancer-cell-specific delivery systems, such as engineered viral or non-viral vectors that are highly tropic and low immunogenicity (Mengstie et al., 2022; Du et al., 2023). Also, CRISPR technology can be further improved (high-fidelity Cas9 variants, base editing, or prime editing) to make it more precise, less risky in off-target effects, and more efficient in editing to become therapeutically relevant (Khan et al., 2019); Ding et al., 2023). The promise of genome level cancer therapy will require the implementation of longitudinal safety studies, as well as careful regulatory frameworks to fully realize the promise safely.

3. CONCLUSION

6.1 Summary of Key Findings

This paper illustrates that CRISPR-Cas9 has the ability to correct mutations in cancer cell models in vitro with a successful percentage of clones showing homology-directed repair (HDR) reversing oncogenic alleles to wild-type. Corrected clones showed functional normalization such as low proliferation, rescue of apoptotic responsiveness and re-balanced signaling pathways which positioned their phenotype closer to non-mutant control cells. Under optimized conditions, off-target analysis indicated that there were only a few unwanted edits. In sum, these results give evidence-of-concept that cancer-specific genomic repair has the potential to reverse malignant cells at the cellular level.

6.2 Significance of Results

The findings highlight the possibility of this paradigm shift in cancer therapy - to chronic inhibition of cancerous signaling to long-lasting genomic repair of disease-promoting mutations. This is in contrast to the traditional approaches of only treating or suppressing a symptom or pathway, and CRISPR-based correction targets the underlying genetic cause of the condition, providing a lasting solution. This high-level genomic remedy would lessen the duty of consistent medication, decrease the drug resistance and enhance the overall effectiveness of the therapy. With the development of genome editing tools and systems of delivery, these outcomes contribute to the far-term perspective of precision oncology based on personalized or individualized genomic therapy, instead of the large-scale cytotoxic or targeted drugs.

6.3 Future scope in Cancer Therapy

In the future, gene editing with CRISPR has the potential to be applied with great potential in clinical cancer therapies. Possible future applications might be CRISPR-based correction of oncogenic mutations alongside immunotherapy, e.g. editing tumour cells or immune cells to increase immune clearance and recognition of the edited cells (Ou et al., 2021; Feng et al., 2024). Moreover, to facilitate safe and efficient in vivo editing of patients, additional optimization of in vivo delivery systems including nanoparticle-based carriers targeted to tumours is essential (Rauf et al., 2025; Li et al., 2023). The combination of these developments could take CRISPR-based cancer therapy out of the laboratory and clinical environment, into the clinic as a new generation of curative and personalized therapies.

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