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CRISPR Functional Screening for Discovering Key Factors in DNA Damage Repair

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Abstract. Traditional genetic screening approaches are limited by low sample throughput and frequent off-target errors, which greatly hinder the identification of critical regulatory factors involved in DNA damage repair. To overcome these technical bottlenecks, multiple CRISPR library screening platforms have been developed and optimized for relevant research. As a classic genome-wide screening technique, CRISPR-KO functions by inducing complete knockout of non-essential genes. However, this method has inherent limitations for DNA repair research, since numerous core DNA repair genes are indispensable for cellular survival. To address this defect, CRISPRi has emerged as a viable alternative, which moderately suppresses gene transcription rather than achieving full gene knockout, thus adapting to the study of essential repair genes. In addition to the two aforementioned tools, Repair-seq adopts a unique technical principle by directly capturing and documenting cellular DNA lesion repair behaviors following DNA damage occurrence. Notably, all these CRISPR-based screening platforms support flexible modification, enabling researchers to tailor them for experimental systems that generate specific types of DNA damage. This review systematically summarizes the technical merits and practical applications of mainstream CRISPR screening platforms for DNA damage repair research, and further highlights the unresolved deficiencies and existing gaps in current relevant studies. Many current studies employ only a single type of DNA-damaging agent. In addition, most candidate genes identified from screening lack sufficient mechanistic validation.

Keywords: DNA damage repair, CRISPR KO, CRISPRi, Repair seq

1. Introduction

DNA stores genetic information for living organisms. Its stable structure maintains normal cell growth, cell differentiation and normal tissue state. Both internal metabolic activities and external environmental exposures continuously induce different types of DNA damage. Base modifications, DNA double-strand breaks (DSBs) and base mismatches are typical examples. Cells accumulate gene mutations and chromosomal rearrangements if they cannot repair such lesions quickly. These molecular changes may further lead to cell aging, programmed cell death, cancer transformation and human inherited diseases. For this reason, studying DNA damage repair mechanisms has great research value. Finding key regulatory genes helps basic biological research and clinical work.

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Defects in the DNA damage response (DDR) make the genome unstable. Genome instability is a main cause of cancer, inherited diseases and aging. Cells are frequently exposed to many stressors such as oxidative damage and ionizing radiation. Among all DNA lesions, DSBs exert the strongest toxic effects on cells. Three major pathways repair DNA double-strand breaks. These pathways are NHEJ, HDR and MMEJ. They overlap and compete with each other during DNA repair. NHEJ does not require homologous DNA templates. It works in the G1 phase and most other cell cycle stages. It rejoins broken DNA ends rapidly. This process easily produces small insertions and deletions, causing repair errors. Genomic DNA damage repair is a coordinated network of multiple pathway systems, each of which has distinct mechanistic properties and functional roles.

The homology-directed repair (HDR) pathway serves as an accurate pathway for genome recovery. It enables error-free genetic restoration by using homologous DNA sequences as a repair template. This high-fidelity repair mode is, however, tightly coupled to the cell cycle progression, and its functional activation occurs during the S and G2 phases of the cell cycle. A subset of proteins, such as ATM, BRCA1 and RAD51, are core regulatory proteins that are essential for the complete and effective execution of the HDR pathway.

In addition to the high fidelity HDR pathway, cells use alternative end-joining pathways to maintain genomic integrity when the primary pathway is unavailable. The primary conventional repair pathway is canonical non-homologous end joining (NHEJ) and the secondary compensatory backup pathway is microhomology-mediated end joining (MMEJ). MMEJ is an alternative pathway of DNA break repair that requires short microhomology sequences near the damaged ends of the DNA. Although it has a compensatory repair ability, MMEJ has relatively low accuracy, with a high frequency of large-scale genomic deletions and has a much higher mutagenic risk than repair with traditional NHEJ [1].

One of the greatest difficulties with elucidating DNA damage response (DDR) networks is the functional redundancy and compensation amongst repair pathways. The vast majority of DDR-related proteins and regulatory genes are activated and/or expressed in specific context-dependent manners. These genes are normally expressed at low levels, but are recruited to sites of DNA lesions once stimulated by damage. Their ability to function is further restricted to distinct cell cycle phases and chromatin contexts. This spatiotemporal specificity makes it very challenging to capture and verify DDR gene functions using conventional experimental tools [1, 2].

Furthermore, DDR biology has two fundamental scientific questions remaining that are not answered to date. Dynamic regulation of the rules that govern the cellular real-time choice of different repair pathways is still unclear. At the same time, the precise spatiotemporal coordination mechanisms among various repair factors have not been systematically characterized. These outstanding issues hinder the development of a comprehensive and sophisticated DDR regulatory framework.

Before the advent of CRISPR technology, traditional techniques like chemical mutagenesis, single-gene knockout and RNA interference were heavily used in DDR functional research. These traditional strategies inevitably have universal technical defects: low throughput, limited candidate genes to be detected, low targeting specificity, serious off-target interference, and insufficient gene suppression. Random mutagenesis also introduces an error-rate into experiments. Moreover, in traditional assays there are ubiquitous screening blind spots due to pathway compensation. These methods are also labor-intensive, have long experimental cycles, lack repeatability, and are not suitable for large-scale genetic screening, which greatly limits the systematic exploration of complex DDR signaling cascades.

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This article systematically reviews the technical principles, advantages, and research progress of various CRISPR screening methods. It also integrates screening results from different DNA damage models and summarizes core DDR genes and regulatory pathways. By addressing the limitations of traditional screening approaches and refining existing research frameworks, this work provides a theoretical reference for research into genome stability, cancer, hereditary DNA repair deficiency disorders, and the safety of gene editing.

2. Various screening tools of CRISPR technology

Conventional DDR research has been bottled up until now by genome-wide screening via CRISPR-Cas9. The technology offers full genome coverage, high-precision DNA targeting and stable high-throughput sequencing, which allows for a thorough study of the critical genes and signaling pathways in the process of sensing DNA damage, adaptation to stress, and repair of lesions. It is consequently the most popular platform for current DDR functional genomics studies [1].

In addition to confirming the canonical roles of extensively studied repair genes such as ATM, BRCA1 and RAD51, CRISPR library screening reveals a large number of new molecules involved in the DDR. CRISPR screening coupled with radiotherapy and chemotherapy-induced damage models is an effective method to discover core genes involved in modulation of therapeutic resistance in tumors. These candidate genes identified from screening are potential biomarkers and targets for improving clinical radiochemotherapy regimens and precision targeted drugs.

The CRISPR-Cas9 editing mechanism is based on the adaptive immune system of bacteria, which protects the host cells against the invasion of phages. sgRNA molecules are used to guide Cas9 nuclease to a specific sequence in the genome to create double-strand breaks (DSBs) in the DNA. These breaks are subsequently mended by endogenous cellular repair pathways, thus completing whole-genome editing. The high-throughput nature of the sgRNA libraries enables the modification of tens of thousands of genes in parallel. Existing mainstream CRISPR screening technologies, such as CRISPR-KO, CRISPRi, and Repair-seq, can achieve permanent gene knockout, moderate gene transcriptional inhibition, and real-time monitoring of break repair in cells, respectively. These flexible platforms accommodate loss-of-function and gain-of-function assays to help researchers discover new and uncharacterized DDR regulatory components [3, 4].

Although CRISPR library screening offers promising revolutionary benefits for DDR research, there are still some significant drawbacks to its use in current applications. The vast majority of previous studies use only one stimulus of DNA damage, and do not compare the behavior of multiple repair pathways in parallel. Moreover, most screening results only provide phenotypic interactions of candidate DDR genes, and there is not enough verification of protein interactions and downstream molecular cascades. A full and integrated DDR regulatory network has yet to be built, in part because high-throughput data describing pathway redundancy and compensatory responses upon damage are still limited.

Advances in editing techniques have rendered CRISPR-based tools essential for DDR research. Commonly used systems are CRISPR-KO, CRISPRi and CRISPRa, combinatorial multi-gene screening and base-editing platforms. Each has its own principles and is suitable for different experiments.

2.1. CRISPR-KO (gene knockout)

Cas9 nuclease makes DSBs at target loci. Cells use error-prone NHEJ. This process creates insertions or deletions. These changes permanently stop target-gene function. Well-established

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libraries give reproducible outcomes. Within DDR research, it is mainly applied to screen non-essential repair genes. Researchers assess cellular sensitivity toward irradiation or chemotherapeutic agents after gene ablation, and uncover candidate genes participating in DSB repair and damage tolerance.

2.2. CRISPRi (CRISPR interference for gene repression)

CRISPRi differs from CRISPR-KO. It employs catalytically dead Cas9 (dCas9). This protein is fused with repressive protein domains. dCas9 will not cut genomic DNA. It is guided to gene promoter regions. It moderately reduces the level of gene transcription. This method produces reversible gene down-regulation. It brings low toxicity to cells. Complete gene knockout may trigger cell death. CRISPRi can prevent such cell death. It fills certain experimental gaps under such circumstances. These gaps cannot be covered by CRISPR-KO for the study of essential repair genes. It works well for exploring dose-dependent functions of core DDR genes such as ATM, BRCA1 and RAD51, as well as dissecting endogenous genetic networks that control DNA damage repair.

2.3. CRISPRa (CRISPR activation)

CRISPRa employs nuclease-dead dCas9 fused with transcriptional activation domains. Guided by sgRNA molecules to gene promoters, this complex elevates target gene transcription without generating DNA cleavage, hence realizing gain-of-function genetic manipulation. CRISPR-KO and CRISPRi suppress or remove gene function; CRISPRa works in the opposite direction by raising transcript abundance. It supports bidirectional experimental validation and reduces analytical bias brought by screening strategies that only knock down or knock out genes. It helps researchers understand how repair gene overexpression shapes genome stability and DNA repair efficiency, while identifying positive modulators that improve cellular repair capacity. In one case, genome-wide CRISPRa activation libraries were used to screen cisplatin-resistant tumor cells. Increased RAD54L expression enhanced homologous recombination activity and produced platinum drug resistance for cancer cells.

This experimental category contains Cas9-based dual or multi-gene knockout, high-throughput Cas12a parallel screening, and SPIDR-assisted combinatorial CRISPRi screening. Researchers perturb two or more genes at the same time. They aim to simulate natural genetic crosstalk. This crosstalk can be observed inside living cells. Combinatorial assays break the constraints of single-gene screening. These assays can directly detect genetic epistasis and pathway compensation. Such experimental platforms show unique value. They help researchers sort out competitive and cooperative relationships. These relationships exist among NHEJ, HDR and MMEJ in the course of DSB repair. For instance, single deletion of BRCA1 only causes partial suppression of homologous recombination. Tumor cell survival decreases dramatically [5]. This outcome occurs when researchers disrupt BRCA1 alongside core MMEJ-related components. This finding offers solid evidence for compensatory effects between repair pathways. It also provides clues for identifying synthetic lethal therapeutic targets.

2.4. Base editors and prime editors

Conventional CRISPR-Cas9 depends on DSB formation to start genome editing. Artificially induced DSBs can activate robust endogenous DDR responses. These responses generate insertions, deletions as well as chromosomal abnormalities. This type of background noise creates obstacles. It

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prevents the accurate reconstruction of clinical pathogenic point mutations. Researchers want to avoid these side effects related to DSBs. They have developed precision editing tools. These tools do not produce DSBs. Two representative tools are base editors (BEs) and prime editors (PEs). Neither system introduces DNA double-strand breaks. Base editors fuse dCas9 with deaminase enzymes. They complete targeted C-to-T or A-to-G base conversions through this fusion. Prime editors recruit reverse transcriptase to directly install desired base substitutions or short insertions/deletions at designated sites. Owing to the absence of DSB-driven widespread repair responses, these editors yield low background signals. They can precisely recreate clinically relevant missense mutations within DDR genes. Researchers can study how single-amino-acid changes affect repair-protein activity and change the choice of DNA repair pathways. Researchers can introduce disease-related missense mutations into the ATM or BRCA2 gene. Related tests check if these mutations impair protein stability, disturb molecular binding and weaken homologous recombination. These results explain the molecular causes of hereditary breast cancer and ataxia-telangiectasia.

2.5. Comparison of different CRISPR derived tools

CRISPR-KO works well for screening non-essential genes by completely removing gene function. It cannot study genes that kill cells after deletion. CRISPRi slightly reduces the activity of essential repair genes. CRISPRa is used for tests of enhanced gene function. Combined screening methods show how different repair pathways make up for each other. Base-editing tools focus on single point mutations. Data collected from these different tools forms a complete technical system to find new DDR genes.

2.6. Limitations and clinical applications of CRISPR screening

CRISPR-based screening has its own limits. Cas9-induced artificial DSBs disturb normal repair phenotypes. Regulators with low expression or strong context dependence are hard to find. Base editors also have off-target errors. Poorly controlled DNA repair helps tumors grow, causes drug resistance, immunodeficiency and early aging diseases. DDR genes found by CRISPR screening help explain why tumor genomes become unstable. They give theoretical support for synthetic-lethal therapy, radiation protection and inherited disease research. These genes have real value for clinical use. Researchers performed genome-wide CRISPR screening in BRCA1-deficient tumor cells. This work found PARP1 as a synthetic-lethal target [6].

3. Comparative analysis of screening outcomes using distinct DNA damage paradigms

Various genotoxic insults induce diverse forms of DNA damage such as DSBs, chemical base adducts, stalled replication forks and DNA interstrand cross-links. Each category of DNA damage triggers specific repair routes. These repair processes rely on separate groups of functional genes. Researchers can adopt CRISPR-KO, CRISPRi, Repair-seq and combinatorial screening to gain multi-dimensional analytical insights. Such approaches support complete gene knockout, moderate transcriptional suppression, multi-gene biological crosstalk study, and characterization of repair products. CRISPR-KO produces consistent phenotypic outputs. This method works for the study of non-essential genes. CRISPRi prevents the generation of external DSBs. This tool enables the investigation of essential genes. Combinatorial screening reveals compensatory biological relationships. Repair-seq characterizes repair-product profiles after nuclease-mediated DNA cleavage.

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3.1. Screening under Ionizing Radiation Induced DSB damage

Ionizing radiation generates many evenly distributed DSBs. It turns on NHEJ, HDR and MMEJ at the same time. It acts as a standard experimental model to study DSB responses. Most screenings in this system use CRISPR-KO and CRISPRi. Results from these two tools support each other. For example, researchers used CRISPR-KO libraries and ionizing radiation on tumour cells. They found non-essential genes for DSB sensing and end-joining. These genes include ATM, PRKDC and LIG4. These genes form a gene set that controls radiation sensitivity. Later CRISPRi tests partly lowered the activity of essential repair genes. Full loss of these genes kills cells. These tests found extra radiation-response regulators. CRISPR-KO cannot find these regulators. Comparing data from both tools greatly increases what the screening can detect [7, 8]. These studies show how different repair pathways work together after radiation damage. They provide candidate genes to make drugs that boost radiotherapy or protect cells from radiation.

Genome-wide CRISPR-KO libraries compare cell survival after irradiation to isolate radiation-sensitive or resistant genes. They enrich non-essential genes participating in DSB sensing, signal transduction and end processing, expanding target pools for radiosensitization and radioprotection. As one illustration, this strategy pinpointed LIG4, a core NHEJ pathway component. LIG4 knockout cells display markedly elevated radiosensitivity, implying pharmacological LIG4 suppression as a promising radiosensitizing approach [8].

A genome-wide CRISPRi work published in Cell Genomics applied low-dose ionizing radiation to preserve cell viability. The dCas9-KRAB system moderately repressed essential DDR genes such as ATR and CHK1. Consequently, many essential DDR factors missed by CRISPR-KO were recovered. In the CRISPRi setup, dCas9-KRAB is recruited to gene promoters by sgRNA. It triggers local heterochromatin formation and achieves reversible, mild transcriptional repression without DSB generation or complete gene knockout, thus preventing cell death caused by total loss of function of essential genes. Gene sets obtained from CRISPRi add extra members of homologous recombination and cell cycle checkpoint cascades to CRISPR-KO results [9]. Partial gene repression mimics physiological endogenous damage response states and mitigates secondary stress artifacts triggered by full gene ablation.

CRISPR-KO is preferred for large-scale primary screening focusing on non-essential repair genes, auxiliary factors and damage tolerance mediators. CRISPRi addresses essential DDR genes such as ATM, BRCA1 and RAD51 and fills blind spots associated with lethal gene deletion. In practice, researchers frequently combine both libraries to obtain a more comprehensive portrait of radiation repair networks.

3.2. Evaluating screening studies using chemotherapeutic drug induced DNA damage

Chemotherapeutic drugs cause base alkylation, interstrand cross-links and replication arrest. The DDR pathways turned on by these toxicants are very different from those responding to radiation-induced DSBs. Cell systems treated with chemotherapeutic drugs are useful experimental platforms to study synthetic-lethal interactions and drug resistance in tumour cells. Cisplatin and mitomycin C are typical examples. They make DNA interstrand cross-links. These damages mainly turn on the Fanconi anaemia pathway and homologous recombination. Hydroxyurea stops ongoing DNA replication and selectively activates signalling chains that depend on ATR kinase activity. One published study from NAR Cancer used CRISPR-KO libraries for DNA damage response [10]. This work uses library screening and chemotherapeutic drug treatment. Researchers identify genes

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including FANCM and REV3L. The loss of these genes makes cancer cells more sensitive to chemotherapy. These genes serve as key targets for synthetic-lethal research.

Cas9 and Cas12a editing produce artificial DSBs. These DNA breaks form mutation patterns different from natural DNA damage. Cell survival data cannot tell the individual function of each DNA repair pathway. Scientists develop Repair-seq to fix these research limits. This tool uses high-throughput sequencing to record DNA changes at cutting sites. It classifies three common DNA mutations, including insertions, deletions and substitutions. It finds core DDR genes that regulate NHEJ, MMEJ and HDR repair pathways.

Repair-seq tests show that the loss of LIG3 reduces microhomology-related DNA deletions. It also changes the activity preference of different repair pathways. This data shows detailed genetic evidence for the competition among DNA repair pathways. The application of Repair-seq optimizes the gene-editing process. The adjustment of repair pathway activity decreases large DNA fragment loss and abnormal gene rearrangement.

3.3. Defining application boundaries of different CRISPR screening modalities

CRISPR-KO applies to non-essential repair genes. It observes cell changes caused by complete gene loss. CRISPRi acts on key DDR genes. These genes support basic cell survival. Repair-seq collects all DNA repair results after nuclease cutting. The three tools have separate functions. They cannot take the place of one another.

4. Conclusion and future perspective

High-throughput screening using CRISPR-KO, CRISPRi, Repair-seq and related tools has found many new DDR regulators and improved our understanding of repair networks that respond to radiation, chemotherapy and replication stress. Modern CRISPR screening tools effectively solve the main drawbacks of traditional screening methods, especially their limited throughput and low resolution. Although current DDR screening technologies have been greatly improved, further optimization is still possible. Combining CRISPRi with Repair-seq can improve the accuracy of DNA damage repair analysis. Meanwhile, better sgRNA design and simpler targeted libraries help reduce sequencing costs and avoid off-target errors.

In the future, DDR screening will adopt more advanced strategies, such as base-editor libraries, organoid verification and in vivo animal screening. Updated bioinformatic tools will also help translate newly discovered DDR targets into real clinical applications more efficiently. However, CRISPR editing still carries potential risks because it produces DNA double-strand breaks (DSBs). Excessive abnormal DSBs cause genomic instability and chromosomal rearrangements, which may lead to cancer. Poor DSB repair is closely related to various human diseases. Mutations in key DDR genes including ATM, BRCA1 and RAD51 increase mutation rates, promote tumor growth and cause drug resistance. In addition, impaired DNA repair disrupts lymphocyte gene rearrangement, slows immune cell development and results in immunodeficiency. Therefore, studying DDR regulatory networks and reducing DNA damage risks in gene editing is theoretically meaningful and clinically valuable. This research helps explain how genomic stability is maintained and improves our understanding of diseases such as tumors and immune deficiency disorders.

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