

# The Application and Prospect of Single-cell CRISPR Screening Technology in Tumor Research

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## Abstract:

Traditional population sequencing methods struggle to directly correlate gene perturbations with transcriptome changes at the single-cell level, due to limitations such as long cycle times and significant batch effects. Single-cell sequencing (SCS) can reveal the diversity and dynamics of cell populations, while CRISPR gene editing and screening technologies enable large-scale functional gene discovery. The combination of these two approaches has led to the development of ScCRISPR-seq. This article reviews the principles of CRISPR screening and SCS technologies, introduces the development of ScCRISPR-seq, and, drawing on research on lung cancer and intrahepatic cholangiocarcinoma (ICC), summarizes its key applications in cancer research, including deciphering tumor heterogeneity, elucidating mechanisms of drug resistance, and modulating the immune microenvironment. This article further explores challenges with this technology, including experimental workflow, data sparsity, and technical noise. Solutions are proposed, including data completion using AI algorithms, eliminating amplification bias using UMI tags, and optimizing gene-phenotype associations through network modeling. However, clinical translation is limited by unclear mechanisms of action and high barriers to entry in drug development. Future research may consider combining ScCRISPR-seq with network pharmacology and molecular docking techniques to improve the efficiency of new cancer drug development and advance precision immunotherapy.

**Keywords:** Gene editing technology, CRISPR screening, single cell sequencing, single cell CRISPR sequencing, tumor.

## 1. Introduction

Tumor is one of the most important diseases threatening human life. Understanding the molecular mechanisms of tumor heterogeneity and its impact on therapeutic response is a key challenge in tumor research. Traditional batch sequencing methods cannot directly correlate gene disturbances with transcriptome changes at the single cell level, and have significant limitations including prolonged experimental cycles and pronounced batch effects.

Single-cell sequencing (SCS) technology is utilized for categorizing cell populations based on variations in expression profiles among individual cells. This method enables a comprehensive analysis of complex cell populations, preventing the masking of single-cell heterogeneity that may occur when analyzing a large number of cells collectively. The fundamental components of SCS include single-cell isolation, amplification of the whole genome or transcriptome, and high-throughput sequencing. By scrutinizing cells individually, this technique uncovers cell diversity, identifies rare cell types, and captures dynamic changes within cell populations. Recent advancements in SCS have significantly enhanced our understanding of the diverse nature of tumor cell populations. CRISPR gene editing technology, considered the third generation of gene editing tools, employs Cas proteins and guide RNA to precisely modify targeted DNA sequences. This technology offers notable benefits such as high precision, cost-effectiveness, and the ability to edit multiple targets simultaneously. CRISPR screening technology, a functional genomic approach based on CRISPR/Cas9, facilitates large-scale and efficient target exploration. This method involves designing numerous guide RNAs (gRNA) to target various genes, enabling the knockout or perturbation of genes on a broad scale in cells or animal models. Subsequently, key genes are identified through phenotypic analysis. Following the identification of crucial genes via CRISPR screening, SCS can be employed to detect expression variances resulting from different gene knockouts in distinct cells. This integrated approach allows for a detailed analysis of the relationship between genetic alterations and observable traits, culminating in the development of ScCRISPR-seq.

At present, ScCRISPR-seq is widely used to analyze tumor heterogeneity, track clonal evolution, and clarify drug resistance mechanisms. However, most of the existing studies are targeted at specific tumors, and there is a lack of a review of the main role of ScCRISPR-seq in cancer research. This paper introduces the basic concepts of gene editing technology and SCS, and systematically reviews the key applications of ScCRISPR-seq in tumor research, aiming to provide reference for optimizing the application

of this technology in tumor research.

## 2. Gene Editing Technology

### 2.1 Development History

Gene editing technology refers to the technology of stimulating the gene editing process by introducing targeted DNA double-strand breaks, and then activating DNA repair pathway of cells to realize site-specific modification, insertion or deletion of target genes. It has evolved through three generations: from early zinc finger nucleases (ZFNs) and transcriptional activator-like nucleases (TALENs) to the third generation CRISPR/Cas system. ZFNs and TALENs rely on protein-DNA recognition modules to achieve targeted cleavage, but they have defects such as complex construction and high off-target rate. CRISPR/Cas system uses gRNA to guide Cas protein to recognize specific DNA sequences, and triggers cell repair mechanism by inducing double-strand breaks (DSB) to achieve gene knockout, insertion or replacement. It has the advantages of high efficiency, low cost and minimized cellular disturbance to cells. So it has quickly become the main technical means to improve the genetic information of animals and plants.

### 2.2 CRISPR Screening Technology

CRISPR/Cas9 is one of the most widely used CRISPR/Cas systems. The main process is sgRNA binding to target DNA through base complementary pairing; then Cas9 nuclease cleaves DNA upstream of PAM sequence, causing DNA double-strand break; finally, gene knockout is achieved by using NHEJ pathway, and gene knockout is achieved by using HDR pathway. CRISPR screening technology is a CRISPR/Cas9-based functional genomic approach, which can be used for large-scale and efficient key technology for target identification. Its core mechanism is to design a large number of guide RNAs to target different genes to knock out or disturb genes on a large scale in cells or animal models, and then select key genes through phenotypic analysis.

At present, CRISPR screening technology plays an important role in the field of tumor research, and several key regulatory genes have been identified. For example, PTP-MT1, a mitochondrial protein tyrosine phosphatase, was found to be highly expressed in liver cancer and mediate tumor cell survival in hypoxic environments by regulating cardiolipin synthesis [1]; Cop1 (E3 ubiquitin ligase) deletion was found to enhance antitumor immunity by regulating macrophage invasion in triple negative breast cancer models [2].

### 3. SCS

SCS breaks through the limitations of cellular heterogeneity in traditional batch sequencing by isolating single cells for high-throughput sequencing and analyzing their genome or transcriptome information. This technology has been developed rapidly since its first application in the animal field in 2009, and the breakthrough in plant research was achieved in corn pollen studies in 2015. Currently, its applications in tumor research are becoming increasingly extensive.

#### 3.1 Technical Process

In recent years, the 10x Genomics platform has become more widely used. The technical process covers several key stages. First, based on microfluidic droplet technology, high-throughput cell separation and labeling was achieved by encapsulating individual cells in oil droplets with gel beads containing unique barcode and UMI sequences. After cell lysis, cDNA synthesis and PCR amplification were carried out by reverse transcriptase, and the library was constructed. Finally, clustering algorithm and pseudo-temporal analysis were used to analyze cell differentiation trajectories, and spatial transcriptome sequencing was integrated to verify the positioning accuracy [3]. The platform's core advantage is enabling multidimensional single-cell analyses, including simultaneous detection of single-cell ATAC-seq and RNA-seq.

#### 3.2 Application

The core advantage of SCS lies in the precise analysis of cell heterogeneity, which allows us to see the cell diversity inside the tumor, enabling identification of malignant driver cells that really drive cancer, while establishing the molecular basis for precision therapeutics. This technique has a broad application prospect in the field of tumor research. Studies have shown that this technology can accurately identify tumor cell subsets and has unique advantages in resolving microenvironments and tumor heterogeneity. LGALS1 (Galectin-1) is a  $\beta$ -galactoside-binding protein that hydrolyzes GM1 to galactose by binding to GM1 ganglioside and recruiting  $\beta$ -galactosidase, providing energy to cancer cells and helping cancer cells resist stress. In addition, it can inhibit T cell activity and promote immune escape from tumor microenvironment (TME). In acute myeloid leukemia (AML) studies, SCS successfully identified a subset of resting stem-like cells associated with chemotherapy resistance, and found that LGALS1 was highly expressed in these cells. Therefore, it can be a potential therapeutic target [4]. In addition, SCS was used to distinguish highly malignant neuroendocrine cells (NEs) from other normal or non-ma-

lignant cells in familial neuroblastoma (FNB) studies. These malignant cells were found to have specific genetic changes, such as an abnormal increase in DNA fragments on the short arm of chromosome 17, and to be exhibited preferential utilization of using arginine for growth and survival [5]. The finding reveals heterogeneity among different cells of the same tumor.

### 4. ScCRISPR-seq

ScCRISPR-seq is an innovative way to study the effects of gene perturbations at single-cell resolution by combining CRISPR gene editing with SCS. Its core is to guide Cas9 nuclease to target specific gene sites through sgRNA, combined with single-cell RNA sequencing to analyze edited transcriptome changes, to achieve high-throughput gene function screening [6]. Traditional CRISPR screening only knows that the candidate gene selected is associated with the phenotype, but it does not know the regulatory network of the gene. ScCRISPR-seq can be considered when you want to screen for genes of interest that produce phenotypes and also want to know the effects of each gene of interest on other signaling pathways. There are currently multiple platforms for this technology. Perturb-Seq developed by Dixit et al. in 2016 realized large-scale parallel ScCRISPR-seq for the first time; CROP-Seq proposed by Datlinger et al. in 2017 further improved throughput by simplifying sgRNA detection process; in recent years, Direct Capture Perturb-Seq and other technologies optimized sgRNA capture efficiency [7]. At present, this technology is mainly used in the field of immune cell function research and TME analysis. Its single-cell resolution provides a powerful tool for parsing gene regulatory networks in complex biological systems. This technique has the following advantages: (1) linking CRISPR editing to cell phenotypes: sgRNA is detected simultaneously with mRNA gene expression in cell populations that have been interfered with by sgRNA libraries, which allows correlation of single-cell expression profiles with corresponding sgRNAs. (2) Improve experimental efficiency: Using sgRNA library to do sgRNA interference on cell population once can obtain thousands of cell sgRNA expression profiles after interference, which greatly improves the experimental efficiency of gene interference experiment. (3) Controllable experiments: Any gene that CRISPR can edit can be targeted, and the cells that are specifically treated can be known only based on transcriptional information. (4) Short experimental cycle: only CRISPR perturbation is needed to produce changes in gene expression, and experiments can be carried out on cells without long-term culture to achieve visible changes in cell phenotype, shortening the time of cell culture. (5)

Reduction of batch effects: experimental groups (sgRNA transfected cells) and control groups (sgRNA-untransfected cells) can be prepared in the same experiment. (6) Simplify the analysis process: SCS analyzes multiple targets (such as hundreds of sgRNAs) and multiple samples simultaneously, and enables multiplexed target-to-cell mapping.

## 5. Application of ScCRISPR-seq in Tumor Research

### 5.1 Analysis of Tumor Heterogeneity

Tumor heterogeneity refers to the existence of different subsets of cancer cells in the same tumor, whose genes and phenotypes exhibit certain differences. These variations lead to divergent tumor growth, metastasis, and treatment responses. Therefore, distinct cancer cell subsets require differential approaches in research and therapy. Fully studying tumor heterogeneity can help us develop targeted drugs to accurately treat different cancer cell subsets, improve efficacy, and provide theoretical basis for individualized treatment. ScCRISPR-seq integrates gene editing and single-cell transcriptome analysis technologies to simultaneously track CRISPR-edited genotype and transcriptome characteristics to reveal the impact of driver mutations on cell phenotypes, providing unprecedented resolution for resolving tumor heterogeneity. Lung cancer, characterized by drug resistance and high heterogeneity leading to elevated mortality, represents a critical challenge in oncology. Owing to its highly heterogeneous and complex microenvironment, coupled with its widespread use in immunotherapy, lung cancer serves as an ideal model for studying tumor heterogeneity. Using ScCRISPR-seq, it was found that sequence similarity family 83 member A (FAM83A) and major histocompatibility complex class II DPB1 chain (HLA-DPB1) are key regulatory genes for lung cancer development. Together, they reveal the heterogeneity of lung cancer malignant degree and are important targets for precise treatment [8]. FAM83A expression product accelerates cancer cell metastasis by activating specific pathways; HLA-DPB1 expression product can participate in antigen presentation to activate cell surface glycoprotein (CD8) and T cells to kill cancer cells and inhibit cancer cell growth. Because these two genes are expressed differently in different tumor cells, the growth rate of cancer cells varies, revealing tumor heterogeneity. Therefore, in the specific treatment, people can select the appropriate treatment mode by detecting the expression of FAM 83A and HLA-DPB1 in different cancer cells.

### 5.2 Study on Mechanism of Drug Resistance

Tumor cells are usually able to resist the lethality of drugs through genetic mutations, epigenetic regulation, efflux of ingested drugs, and activation of some compensatory signaling pathways to acquire drug resistance. ScCRISPR-seq has shown great potential in studying the mechanism of tumor drug resistance. By targeting genes associated with drug resistance, SCS is then used to monitor changes in DNA levels, RNA levels, protein levels and phenotypes, and to explore changes in gene regulatory networks and signal transmission pathways of drug-resistant clones to reveal key genes and molecular mechanisms associated with tumor drug resistance. At present, the mechanism of drug resistance in lung cancer is extensively studied. SLFN11 is a DNA/RNA helicase, and studies have found that tumor cells are more sensitive to DNA damaging drugs when their expression levels are high. Poly(ADP-ribose)polymerase (PARP) is a closely related enzyme that recognizes DNA single-strand breaks to recruit repair proteins for repair. Thus, deletion of SLFN11 gene leads to PARP inhibitor resistance by interfering with DNA damage repair pathway. The specific mechanism is that SLFN11 protein deletion allows tumor cells to escape S phase arrest and attenuate PARP inhibitor induced DNA damage accumulation, thus maintaining cell survival [9,10].

Therefore, upregulating SLFN11 expression to augment PARP inhibitor-induced DNA damage accumulation may represent a potential therapeutic strategy for overcoming lung cancer drug resistance.

### 5.3 Immune Microenvironment Regulation

The core objective of studying the TME is to alleviate immunosuppressive effects (such as regulatory T cells), enhance the function of effector cells (such as cytotoxic T cells) to inhibit tumor growth, and optimize the synergy between immune cells. ScCRISPR-seq, utilizing SCS, can analyze the key regulatory genes of immune cells that really play a role in TME after modifying potential target genes, providing precise intervention targets for precise treatment.

Intrahepatic cholangiocarcinoma (ICC) is a very dangerous tumor. Its five-year survival rate is low, merely 23.6%, while the recurrence rate reaches 50%-60%. It seriously threatens human life [11]. It also has a relatively clear immune suppression mechanism and immune infiltration characteristics (the number, distribution and functional status of various immune cells) are readily detectable. Therefore, ICC serves as an informative model for the study of ICC. IKAROS and ETS1 are key proteins that regulate immune cell function. Using ScCRISPR-seq,

IKAROS (IKZF1) and ETS1 were identified as two key regulatory molecules in the microenvironment of ICC [12]. IKAROS transcriptionally regulates immune checkpoint molecules including PD-1, mainly affecting T and B cell development and antitumor activity [13]. Its absence can lead to dysregulation of immune cell function. ETS1 regulates T cell activation and macrophage polarization, thereby affecting the immunosuppressive state of the TME [14]. Synergistic action of both can enhance anti-tumor immune response.

These two molecules maintain T cell differentiation balance by regulating different transcription factor networks to maintain an inhibitory microenvironment against tumor growth while providing new therapeutic targets for immunotherapy [15,16].

## 6. Discussion

### 6.1 Difficulties and Challenges

ScCRISPR-seq provides an unprecedented perspective for analyzing tumor heterogeneity, elucidating drug resistance mechanisms and regulating immune microenvironment. However, the complex workflow from sample preparation through data analysis, particularly the processing of massive sequencing datasets, poses significant technical challenges, and there are still many difficulties and challenges. The biological characteristics of gene expression and technical limitations resulting in data sparsity and technical noise interference are the core challenges facing ScCRISPR-seq. The scarcity of data is mainly caused by the dropout phenomenon: due to the extremely low RNA content of a single cell, many low-abundance transcripts cannot be captured, resulting in false positives; the gene is not expressed at a constant speed in time, but stochastic burst-like expression patterns, resulting in inaccurate data measurement; during the experimental operation, inefficient reverse transcription and cDNA amplification lead to RNA loss. Technical noise predominantly stems from multiple sources: inadequate RNA release due to incomplete cell lysis; systematic errors due to batch differences and contamination of enzyme reagents (such as reverse transcriptase, DNA polymerase); contamination of environmental RNA; and stress reactions during cell sorting. The sparse data and noise interference caused by these factors seriously affect the evaluation of CRISPR editing effect and the accuracy of functional genomics analysis [17].

In addition, there are challenges to be solved in this technology, such as the capture efficiency is limited by the isolation method; the spatial position of cells in tissues is lost; the off-target effect is uncontrollable; the proportion

is unbalanced during the amplification process; discrepancies between gene knockout and phenotypic manifestations.

### 6.2 Future Expectations

To address the technical challenges of ScCRISPR-seq, this paper implements computational approaches to impute missing data, filter noise signals, and perform cross-validation of core hypotheses, proposing the following solutions: (1) For data sparsity, Implement AI algorithms (such as MAGIC) for missing data imputation. When gene expression cannot be measured directly, imputation can be performed based on transcriptional profiles of neighboring cells in the feature space. (2) To resolve the imbalance of amplification ratio and technical noise, UMI tags were used to eliminate PCR errors. After amplification, reads with the same UMI are pooled together as one count. That is, the number of original RNA molecules is equal to the number of different UMIs to eliminate duplication techniques caused by PCR overamplification bands. (3) To resolve gene-phenotype discordance for detailed network construction: Apply GAT-GCN modeling with AI-driven network analysis to identify critical regulatory nodes. Quality control pipelines (such as Seurat) can filter out low-quality cells exhibiting editing artifacts or measurement failures, retaining only successfully edited cells with complete datasets for analysis[18, 19].

## 7. Conclusion

This paper reviews the principle of CRISPR screening and SCS technology, and introduces the development of ScCRISPR-seq. Drawing on the existing studies on some key regulatory genes of lung cancer and ICC, this paper future summarizes the main roles of ScCRISPR-seq in tumor research: analyzing tumor heterogeneity, clarifying drug resistance mechanism and regulating immune microenvironment. It proves that ScCRISPR-seq is one of the revolutionary tools in tumor research. In addition, this paper proposes potential strategies to address the current limitations of ScCRISPR-seq. The results of this paper can provide multi-dimensional analysis perspectives including drug resistance and heterogeneity for tumor therapy, and provide references for researchers to select technologies and optimize methods to optimize precise immunotherapy of tumors. However, due to unclear target mechanisms and high clinical trial thresholds, the pharmaceutical industry is afraid to develop potential therapeutic drugs, resulting in low clinical conversion rates in this field. In recent years, network pharmacology and molecular docking technology have developed rapidly, with the functions of rapidly screening potential active ingredients

and predicting drug-target interactions, which can significantly improve the efficiency of cancer drug development. Future research could consider combining ScCRISPR-seq, network pharmacology, and molecular docking to greatly enhance the effectiveness of new drugs.

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