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Functional CRISPR Screening in Breast Cancer: Gene Discovery and Therapeutic Strategies

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Abstract. Although there have been some achievements in early detection and treatment, breast cancer is still a leading cause of cancer deaths among women worldwide. Due to differences in genes, signals, and how well they respond to treatment among the various types of breast cancer, there is frequently recurrence or resistance to therapy. The old candidate gene method and the RNAi screening method lack a priori knowledge and do not cover all gene interactions in the search for unknown gene regulatory factors. CRISPR-based functional genomics screening is a good way to study the functions of all the genes in the genome systematically. Both loss-of-function and gain-of-function types of CRISPR screening can be employed to find tumour-driving genes, drug-resistance factors and other good therapeutic targets. CRISPR has been applied to the screening of breast cancer in the discovery of driver genes, endocrine therapy resistance, chemotherapy resistance and other targeted therapy strategies. Problems and Future Directions of CRISPR Screening in Precision Medicine for Breast Cancer at Present are listed below.

Keywords: CRISPR screening, Breast cancer, Functional genomics, Drug resistance, Precision medicine

1. Introduction

Breast cancer has many molecular subtypes, such as ER-positive/HER2-negative, HER2-positive and triple-negative breast cancer (TNBC), which are caused by different genes, use various signalling pathways and have different drug responses. ER-positive breast cancer is caused by estrogen receptor signalling and treated with tamoxifen or other endocrine therapy; HER2-positive cancer results from HER2 amplification and is treated with trastuzumab. TNBC does not have ER or HER2 genes and is therefore difficult to treat. The previous treatment will probably result in drug resistance and recurrence of the disease; thus, the identification of new essential regulatory genes as therapeutic targets is necessary. The two kinds of traditional research are: First, one needs to learn about specific candidate genes that are believed to be involved in cancer; for instance, knocking down a gene can be done to observe changes in cells, but this method is limited to known genes and depends on existing knowledge. It is also not possible to study only a few genes at a time and fail to find any new regulatory factors. The second is RNAi screening (siRNA or shRNA), and it has a significant limitation: RNAi only reduces the amount of mRNA but does not eliminate the gene. RNAi often has a false-negative result for genes with low expression or for proteins that have a long

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half-life because these proteins can still be produced after reducing mRNA. CRISPR technology has some advantages over the former ones. CRISPR-Cas9 knockout screens, as well as CRISPRa (activation) and CRISPRi (interference), are all kinds of genetic screens that work by deleting genes. A library of sgRNAs is used to generate various gene knockout conditions in the cancer cells. Subsequent sequencing of sgRNA shows that the absence of a particular sgRNA means the corresponding gene is necessary for cell life. CRISPRa is employed to increase the expression of genes that promote the survival of cancer cells, and CRISPRi is used to reduce gene expression and mimic gene downregulation. Finally, CRISPR-Cas9 will cause irreparable damage to the DNA and thus lead to a complete loss-of-function of the gene. Moreover, RNAi will only decrease the quantity of mRNA (partial knockdown) and is subject to off-target effects. CRISPR technology is now employed in research to find genes that cause the development of breast cancer and also in the genes that inhibit it. CRISPR is also related to therapy resistance. It can help find out which gene deletions or activations cause the development of drug-resistant models. Finally, CRISPR has been applied therapeutically and synthetic lethality has been discovered [1]. CRISPR screening can be used to find the genes that cause the resistance to endocrine therapy in the above cases. Hany carried out experiments on ER-positive breast cancer cells, built a genome-wide CRISPR-Cas9 knockout library, selected cells after tamoxifen treatment, and thus found that the purine biosynthesis pathway affects ERα activity and the response to tamoxifen [2].

CRISPR screening in breast cancer research has been used to find many new biological processes that were previously unknown through other methods. Whole-genome CRISPR technology has been employed to find genes involved in various cancers, explore the regulation of epithelial-mesenchymal transition (EMT) networks, uncover mechanisms of resistance to endocrine therapy and chemotherapy in cells, and discover new drugs for these diseases. CRISPR-Cas9 screening has found new functions for the open reading frames of long non-coding RNAs (lncRNAs) in human cancers, and it has also been shown that non-recessive genomic elements are involved in the biological processes of tumours [3]. CRISPR-Cas13d screening has also discovered KILR, an lncRNA that is involved in DNA replication and repair in cancer, and it has been shown that CRISPR methods can be employed to investigate non-coding regions that are difficult to study by other means [4]. CRISPR screening has found new genes and also been employed to investigate the origin of drug resistance. Hany and others have employed CRISPR screening to inhibit the proliferation of cancer cells in the problem of endocrine therapy resistance [2]. CRISPR-Cas9 whole-genome screening was carried out in the ER-positive breast cancer model treated with tamoxifen, and it was found that the purine biosynthesis pathway is required for the activation of Estrogen Receptor alpha (ERα) and tamoxifen resistance. CRISPR screening has found genes in the metabolic pathway that affect the response to treatment in this study [2]. In the same way, other causes of drug resistance have also been identified, such as the function of Tafazzin in regulating phospholipids in tamoxifen-resistant ER-positive breast cancer cells [5].

Although CRISPR-based screening has made some progress, it is still facing many problems that prevent widespread use in daily life. At present, most of the methods are in vitro systems based on reductionism, and they cannot fully replicate the complexity of the tumor microenvironment, such as cell crosstalk, immune regulation and metastasis. The genes that can be obtained in this way are not necessarily good therapeutic targets, so further functional validation in preclinical animal studies and subsequent clinical trials need to be conducted to determine their translational value.

Review of the Current Status of CRISPR Technology in Cancer Screening. First, this review examines how CRISPR screening has been employed to identify oncogenic drivers and regulatory elements in cancer. Second, there is also the issue of endocrine therapy resistance and chemotherapy

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resistance. Finally, this review discusses new therapeutic targets and combination therapy strategies identified through CRISPR screening, as well as the challenges that remain in clinical translation.

2. CRISPR screening in breast cancer driver gene discovery

2.1. Identifying coding and non-coding genetic drivers through CRISPR screening

The goal is to find genes that are responsible for causing or promoting the growth of cancer. Most of the previous genomic studies have focused on regions of genes that are frequently altered, and many other functional regulatory elements have not been found by this method yet. CRISPR-based functional screening is an unbiased way to address this problem, and one can directly know how much a single gene or a section of DNA affects the fitness and survival of cancer cells. Genome-wide interference can be used to find the first set of cancer-related genes and new regulatory factors in the biology of breast cancer (Table 1).

Another good point of CRISPR screening is that it can reveal the function of a region in the genome that does not code for proteins. Although most of the previous cancer research has focused on annotating mutations in coding regions, more and more evidence has shown that non-coding areas can also regulate the development of tumours in various ways. Using a conventional CRISPR-Cas9 screening method, Zheng et al. identified several long non-coding RNAs (lncRNAs) containing potential open reading frames (ORFs). Research has shown that some lncRNA-derived ORFs can produce functional peptides involved in human cancer biology, so it is no longer assumed that most non-coding transcripts are not protein-coding [3]. Therefore, the end result is that CRISPR screening can find some functional elements that are difficult to discover through other means and extend the range of discovery of cancer drivers.

CRISPR screening can be used to identify previously unrecognized genetic elements and investigate the functions of breast cancer-related non-coding RNAs. Wang and others employed CRISPR-Cas13d screening to find lncRNAs that are involved in the process of breast cancer cells and others. Therefore, KILR has been classified as an lncRNA in breast cancer that regulates the DNA replication and repair pathway [4]. Changes in the processes of DNA replication and repair can affect the stability of the genome and thus influence tumour evolution and drug response. KILR has been identified, and with the help of a CRISPR-based RNA screening method and the CRISPR method, the regulatory function of non-coding transcripts can be investigated by targeting DNA.

2.2. CRISPR screening for genes regulating tumor survival and metastatic progression

Many cancer cells use certain genes to promote cell division, cope with stress and survive. CRISPR screening has been employed to find protein-coding genes that control the life and spread of breast cancer cells, and coding sequences have also been analysed. This analysis can identify genes that may serve as potential targets for new drug development.

Einstein and others have used a CRISPR-based screening method to find the sensitivity of MYC-driven breast cancer. YTHDF2 is a typical factor that promotes the survival of cancer cells; therefore, it is expected that the inhibition of YTHDF2 will lead to MYC-mediated proteotoxic stress and cell death in breast cancer cells [6]. Overexpression of MYC increases the demand for protein synthesis and metabolism in the cell, so it becomes dependent on the machinery of protein homeostasis. CRISPR disruption of the YTHDF2 gene has reduced the sensitivity of MYC-driven tumours to drugs. CRISPR screening has obtained a particular result that was not achieved in the previous molecular tests, and this paper reports on this.

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CRISPR screening has been employed to find genes in the epithelial-mesenchymal transition (EMT) pathway that are associated with cancer invasion and metastasis. Zhang and others performed a genome-wide CRISPR screen to find regulators of all EMT pathways and discovered that PRC2 and KMT2D-COMPASS complexes regulate different states of EMT and have different effects on metastasis [7]. Based on the above findings, different regulatory pathways can result in various cellular states and promote metastasis through different mechanisms; thus, EMT is not a uniform process. The above results show that CRISPR can be used to study many diseases and find particular genes that cause various types of cancer.

In short, the studies mentioned above have shown that CRISPR screening is transforming the identification of cancer drivers. CRISPR screening does not need to know about candidate genes or their correlation with expression beforehand; it can directly find out which gene modification leads to cancer. Unbiased functional genomics will increase the list of cancer driver genes and provide a complete genetic map of the origin, development and spread of tumours (Table 1).

3. CRISPR screening for mechanisms of endocrine therapy resistance

Endocrine therapy is a very good option for ER-positive breast cancer, but over time, many people have developed resistance to this treatment and can no longer take it effectively. Many kinds of endocrine resistance have been observed, such as changes in ER signalling and metabolic adaptation, etc., but many of the genes involved in this resistance are still unknown. Drug resistance generally occurs as a result of multiple pathway disruptions. Therefore, an unbiased and comprehensive identification of the genes that allow cancer cells to survive endocrine treatment is needed.

CRISPR screening is used to find the causes of endocrine resistance by exposing the genome to many kinds of genetic modifications. CRISPR-Cas9 screening will be used to find genes that are associated with the resistance of cancer cells to treatment and to identify genes that affect the sensitivity of cancer cells to endocrine therapy. This approach may reveal previously unknown biological pathways involved in treatment failure.

Hany and others carried out a genome-wide CRISPR-Cas9 knockout screen in an ER-positive breast cancer cell line after tamoxifen treatment to find genes that cause endocrine resistance. Based on the research, genes in the purine biosynthesis pathway are required for the function of estrogen receptor alpha (ERα) and are associated with tamoxifen resistance [2]. Mechanistically, this paper shows that metabolic regulation is associated with hormone receptor signalling and thus cancer cells can evade the effects of endocrine therapy through metabolic adaptation. Therefore, to improve the response to endocrine therapy, one should regulate some metabolic pathways.

Changes in the composition of cell membranes and lipid metabolism are also related to endocrine resistance and other metabolic diseases. Li and others studied the function of tafazzin (TAZ) in ER-positive breast cancer and found that it increases tamoxifen resistance by changing the composition of cellular phospholipids [5]. Therefore, the regulation of lipid metabolism is probably involved in the development of drug resistance. In short, the above studies have discovered new mechanisms of drug resistance that are not related to the traditional hormone signalling pathway, and thus a new target to address the failure of endocrine therapy has been found unnoticed so far. All the above studies have shown that endocrine resistance is also linked to changes in estrogen receptor signalling. Therefore, other ways to help the cancer cells survive are the increased synthesis of purines and modification of phospholipids. In the future, CRISPR screening and metabolomics will be combined to find more resistance-associated metabolic networks and develop metabolism-targeting combination therapies (Table 1).

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4. CRISPR screening for identification of chemotherapy-sensitizing targets

Chemotherapy is still a good choice for severe forms of breast cancer, such as triple-negative breast cancer, when there are no other suitable treatments. Chemoresistance is still a problem because drug-resistant tumour cells can avoid the harmful effects of chemotherapy by changing their metabolism, regulating stemness, modifying DNA repair and stress-response pathways, etc. Therefore, identifying genes that regulate chemosensitivity is necessary for the development of effective combination therapies. CRISPR screening can be used to find genes in the mechanism of chemotherapy that are under selection pressure by the drug throughout the genome. CRISPR screening can find new and previously unknown regulators of drug sensitivity that have gone unnoticed in the previous studies of individual resistance genes.

Yan and others have carried out genome-wide CRISPR screens both in vitro and in vivo to find the cause of paclitaxel resistance in triple-negative breast cancer [8]. Based on the comparison of genetic dependence at different times in the experiment, some regulators of cancer stemness and chemotherapy resistance have been found [8]. Cancer stem-like cells are thought to be a cause of treatment failure because they can resist chemotherapy and generate new cancer cells after treatment. Based on the above results, CRISPR screening has found some genes in the resistant cell line that are expressed more frequently.

CRISPR screening has also found some genes that are linked to paclitaxel resistance and other kinds of resistance to targeted cytotoxic drugs. Chandrasekaran and others have found that USP32 increases the resistance of YM155 to ER-associated degradation of the solute carrier protein SLC35F2 [9]. SLC35F2 is needed for the uptake and cell response of YM155, so USP32 reduces the stability of SLC35F2 to decrease drug sensitivity. CRISPR-based methods can be used to reveal the regulation of protein stability and intracellular drug metabolism that is not known from other expression analysis methods.

In short, CRISPR screening has identified many genes that are associated with chemotherapy resistance and involved in stem cell characteristics, metabolism, protein regulation, drug response, etc. Yan and others have put forward that the stemness pathway can restore the sensitivity to paclitaxel, and Chandrasekaran and others have proposed that blocking the USP32-SLC35F2 axis can overcome YM155 resistance [8, 9]. These findings suggest that combining chemotherapy with stemness inhibitors or protein-stability regulators may improve anticancer efficacy (see Table 1).

5. CRISPR screening for novel targeted therapies and combination strategies

Targeted therapy can improve the prognosis of some types of breast cancer, but it is often ineffective due to the development of resistance. Cancer cells can also activate other signalling pathways to avoid the effect of targeted drugs, increase the activity of survival pathways, or change protein stability. Therefore, it is necessary to determine which genes are associated with sensitivity or resistance to targeted therapies in different patients in order to develop effective combination treatment strategies. CRISPR screening will be used to find genes involved in this process in cancer cells, and then it will be determined whether disrupting these genes reduces the survival of the cells in the presence of treatment.

Triple-negative breast cancer (TNBC) is a difficult type of cancer because it does not have the ER or HER2 genes and has fewer targets. Many signaling pathways have been identified as possible drug targets, but the reason for drug resistance in TNBC cells is often due to all sorts of regulatory mechanisms that help the cells survive. CRISPR-based screening can identify compensation mechanisms and determine which deficiencies require pharmacological correction.

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Ang and others have employed functional genomics to study the regulation of EGFR signalling in lower-grade gliomas (LG) and other cancers [10]. New coordinated regulation mechanisms for EGFR and CDK12/13 have been discovered that control the expression and degradation of oncogenes to promote the growth of triple-negative breast cancer (TNBC) cells. Therefore, it has been determined that a single treatment for one node is not necessary, and flexibility can be achieved through a close-knit network of signals. CRISPR screening data have been used to construct a map of genetic vulnerabilities, and now several genes in the same pathway can be treated together in treatment plans.

CRISPR-based functional screening can find other molecular changes that reduce the effect of targeted therapy by mapping changes in pathways. Cruz Gordillo and others have investigated the inherent and adaptive resistance to EGFR inhibitors in TNBC, and found that ELP-mediated upregulation of MCL1 is a way to bypass this effect and help the cancer cells survive in the presence of EGFR blockade [11]. MCL1 is a type of anti-apoptotic BCL-2 protein, so an increase in the expression of MCL1 will suppress the pro-apoptotic signal and prevent EGFR-induced cell death. CRISPR has studied gene dependence and found some background resistance pathways; thus, a way to rationally combine therapy has been obtained, such as co-inhibition of EGFR and MCL1 or their upstream regulators.

In short, the above studies have shown that CRISPR screening can uncover all sorts of intricate genetic interdependencies affecting the results of treatment. Map adaptive bypass pathways, context-specific synthetic lethality and molecular drivers of treatment evasion, to rationally design synergistic combination schemes that can overcome the inherent and acquired drug resistance in cancer with CRISPR-based functional genomics. Unlike conventional pathway-based drug discovery methods, the combination strategies discussed in this review are based on functional genetic dependency data obtained through CRISPR screening and therefore represent more rational and precise therapeutic approaches (Table 1).

Table 1. Typical CRISPR screening studies

Research focus	Major finding	Potential application	Ref.
Driver gene discovery	Identified functional lncRNA-derived ORFs and KILR involved in DNA replication and repair	Discovery of novel breast cancer drivers	[3, 4]
Drug resistance	Revealed metabolic adaptation, lipid remodeling, and stemness-associated resistance mechanisms	Development of sensitization strategies	[2, 5, 8]
Targeted therapy	Identified genetic dependencies supporting combination therapy	Precision targeted treatment	[10, 11]

6. Conclusion

CRISPR-based functional screening has changed the focus of breast cancer research from candidate-driven studies to unbiased genome-wide functional studies and directly shown the causal relationships among genetic alterations and the disease.

In the discovery of breast cancer drivers, CRISPR technology has increased the scope of research on gene regulation to non-coding genes, and new targets such as lncRNA-derived peptides and oncogenic lncRNAs that promote the growth and spread of cancer have been found. CRISPR screening of endocrine therapy and chemotherapy resistance has identified the metabolic pathway, lipid regulation mechanism, stem cell-related factors and protein stability regulator that lead to treatment failure. CRISPR screening has been used to find compensatory signalling networks in the

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face of drug resistance to targeted therapy, and it can also guide the development of rational combination therapies.

Although there have been some improvements, there are still some problems with applying CRISPR screening to clinical precision medicine. First, many of the current screening methods are based on simplified cell culture systems and do not fully represent the tumour microenvironment, such as the interactions among cancer cells, immune cells and the extracellular matrix. Second, the genetic dependence identified in the screening must be further verified, as the hit of screening may not be the desired clinical treatment goal. Finally, current in vivo screening methods still have limitations. The clinical translation of laboratory findings may be improved by combining CRISPR screening with single-cell sequencing, spatial analysis, and patient-derived models.

In the future, CRISPR screening will be used to increase the biological relevance and screening accuracy of CRISPR, thus advancing the development of gene discovery and therapy. Functional genomics and new technologies are being combined to carry out CRISPR screens for personalised medicine in cancer and improve the prognosis of patients.

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