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Review

What Does CRISPR Technology Provide to Cancer Treatments?

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ABSTRACT

Today, cancer is the second leading cause of death in the world after cardiovascular disease. When the carcinogenesis formation processes are examined, we consider that many theories are trying to explain this. These theories vary widely in terms of both origin and predisposing causes in terms of genetic, epigenetic, immune system origin or monoclonal antibody theory. Starting from the DNA damage in the division stage of the cell, the mutated cell's escape from apoptosis, gaining unlimited proliferation capability with telomerase activity, angiogenesis ability, metastasis, invasion, escape from tumor suppressor genes, managing tumor microenvironment, tumor progression, and heterogeneity features is a multi-parameter complex equation. While trying to explain the mechanisms of carcinogenesis, many unknown pathways and biomarkers also emerge. Clustered regularly interspaced palindromic repeats/CRISPR-associated9 (CRISPR/CAS9) technology for cancer biology has the potential to respond to many unknowns at the cellular and genetic level. With this article, we aimed to predict the promise of CRISPR/CAS9 technology in the field of oncology.

Keywords: Cancer, CRISPR, CRISPR/CAS9, drug development, oncology, treatment.

HISTORY OF CRISPR/CAS RESEARCH

The discovery of CRISPR (Clustered regularly interspaced palindromic repeats) sequences started with Escherichia coli studies in 1987.[1,2] The first scientist who enlightened the CRISPR loci in 1993 was Francisco Mojica from the University of Alicante in Spain.[3] We learned the structures of the CRISPR series that we know now in 2000. And in 2007 it was discovered that viruses are responsible for the defense system against phages. The discovery in 2005 that CRISPR has the same homology as the genome of bacteriophages further characterized the CRISPR sequences and it was determined that CRISPR is a unique form of the bacterial adaptive immune system.[4]

Again in 2005, Alexander Bolotin discovered the CAS (CRISPR-associated proteins) gene set, which is predicted to have nuclear activity and encode a large protein named Cas9 (CRISPR-associated9) at the CRISPR locus of Streptococcus thermophilus.[4] In 2006, Eugene Koonin demonstrated a basic structure for CRISPR steps based on the addition of homologous sequences to the bacteriophage genome in the differential sequence.[5] The hypothesis was established in 2007 that the target bacterium would develop resistance against continuous bacteriophage attacks and that viral DNA was added to the bacterial CRISPR region, and if these viral DNA fragments were removed, the bacterium would become sensitive and lose resistance. Subsequently, Philippe Horvath conducted the first experiment with CRISPR systems in S. thermophilus.[6] He studied the insertion of the new phage DNA sequence into the CRISPR sequence and the immunity of bacteria to the next phage attack. At the end of the experiment, it was seen that the passive state of the CRISPR cascade was responsible for phage attacks, while Cas9 protein was responsible for intervention.[7,8] In 2008, the presence of separating sequences of CRISPR RNAs (crRNA) in the genome of E. coli was confirmed. In 2009, different types of

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CRISPR were discovered that could only target RNA. And in 2011, trans-activating CRISPR RNA (tracrRNA) was discovered while sequencing RNA with Cas9 protein determination on Streptococcus pyogenes. It was elicited that the tracrRNA forms a duplex with the crRNA that directs Cas9 to its targets.[9]

The type II system of the CRISPR-Cas locus of S. thermophilus was cloned and as a result, it was determined that this strain has the ability to confer plasmid resistance in E. coli. All these studies have proved that CRISPR systems are autonomous arrays. The first in vitro experiment was conducted in 2012, demonstrating that CRISPR can be designed for targeted DNA fragmentation. In 2013, the first application of CRISPR-based editing in human and mouse cell lines was made.[10] The results of the studies revealed that excessive diversity in the CRISPR mechanism with respect to unique Protospacer adjacent motif (PAM) sequences and types/subtypes of Cas proteins were found in 95% of archaea and 48% of bacterial genomes.[11]

WORKING MECHANISM OF CRISPR/CAS SYSTEM

The CRISPR/Cas system is a special RNA/protein complex developed from the adaptive immune system of bacteria and archaea and is the latest addition to genome engineering tools.[12] Although the CRISPR sequences and Cas genes differ greatly among microbial species, the CRISPR locus; It has a universal anatomy with palindromic repeat sequences, spacer DNA regions, the absence of ORF-Open reading frame for the leader sequence, and the presence of Cas genes.[13]

Palindromic repeat sequences: Although the sequence length and sequence content of palindromic repeat sequences at a particular CRISPR locus is largely preserved; It can differ between species. The lengths of the repeat sequences range from 24 to 47 base pairs (bp), and often contain short 5 to 7 nt palindromic repeat sequences. Palindromic sequences are stable, highly conserved sequences that contribute to the formation of the RNA stem-loop structure (hairpin). Repeat regions in the CRISPR region can be several or hundreds.[14,15]

Spacer DNA regions: Repeat sequences are separated from each other by specific unique sequences called "spacer DNA". Being hypervariable elements of the CRISPR locus, these sequences range in length from 26 to 72 bp. The source of spacer DNAs are nucleic acids of plasmids or viruses. These proto-spacings of 14-16 bp are typically adjacent to conserved short 2-5 nt PAM sequences.[14,15] The spacer DNA locus in a genome can be single or more. These spacer DNAs are the central component of the adaptive immune system in prokaryotes; They consist of specific DNA sequences and form the memory of the acquired immune mechanism. Spacer DNA sequences functioning as a specialized genetic memory are responsible for preventing viruses with recognition sequences from infecting the host.[14-16]

Leader series: The leader sequence is an uncoded sequence rich in Adenine and Thymine nucleotides located at the 5 'end of the CRISPR locus containing about 500 nt; hence it is the starting point of transcription. The leader sequence has no ORF and is not preserved across species. Spacer DNAs that will be newly added to the CRISPR locus are added from the proximal end where the leader sequence is located.[14-16]

Cas genes

Cas genes are usually located near CRISPR sequences and encode Cas proteins. Cas proteins can open and cut DNA sequences owing to their endonuclease, exonuclease, helicase properties and their nucleic acid binding sites (domains). Cas genes are involved in various steps of CRISPR-mediated immunity, such as the integration of spacer DNAs into the CRISPR locus, transcription and processing of crRNAs, directing crRNAs to target DNA, and consequently degradation of the invading target DNA. In 2002, Jansen et al.[17] Identified the first 4 Cas genes, and in the following years, Haft and Makarova study teams increased this number to over 45. CRISPR/Cas systems, according to the genetic components and the difference of Cas genes, three main and 11 subtypes (IA/IF, II-A/II-C, III-A/III-B), which are type I, type II and type III classified as.[14-16,18,19] The CRISPR system is sufficient on its own to trigger double-stranded DNA breaks that can be repaired by non-homologous end-joining (NHEJ) or homology-directed repair (HDR).[18] However, the efficiency and specificity of the CRISPR system is not based on DNA repair mechanisms. In the NHEJ repair mechanism, the DNA ends are chemically linked by a small insertion or deletion at the break site. Therefore, the NHEJ mechanism is often used in the case of gene disruption (small deletions or insertions), inversions, duplications, or deletions, while the HDR repair mechanism is used for large deletions, base mutations, insertions, and substitutions. In the HDR repair mechanism, a donor

DNA molecule pairs with the genomic sequence surrounding the double-strand break (DSB) region, thus introducing new genetic information to the genome at the break site.[18,19] The CRISPR technique can use the HDR mechanism using single-stranded DNA oligonucleotides to cause silent mutations, thus allowing the expected phenotype to be traced in a particular cell type. In particular, the repair pathway is chosen according to the stages of the cell cycle; While NHEJ mechanism is used in cells in G1, S, and G2 phases, HDR mechanism is limited to S and G2 phases.[15,17,20]

CANCER TREATMENT MODALITIES

Today, cancer is the second leading cause of death worldwide, after cardiovascular disease. When we look at the causes of carcinogenesis, we see that there are many theories. Genetic, epigenetic, immune system-derived or monoclonal antibody theory varies widely both in terms of origin and predisposing causes.

Starting from the DNA damage in the cell cycle, the mutated cell's escape from apoptosis, gaining unlimited proliferation capability with telomerase activity, angiogenesis ability, metastasis, invasion, escape from tumor suppressor genes, management of tumor microenvironment, tumor progression and heterogeneity components are special features of the multi-parameter equation for cancer formation. Therefore, treatment models also differ according to the type of formation way of cancer. In short, it is the uncontrolled growth and division of cells, and malignancy is a disease associated with long-distance metastasis of cells.

They are classified according to the tissue affected by cancer, such as breast cancer, lung cancer, skin cancer, and the cell from which cancer originates, such as carcinoma and adenocarcinoma. For example, carcinomas are cancers that originate from epithelial cells that make up a large percentage of cells within the tissue and are the most common type of cancer. It has categories based on characteristic differences. While carcinomas that produce mucus and similar fluid are classified as adenocarcinoma if they originate from breast or colon glandular tissue. Squamous cell carcinomas originate from squamous cells located in the outer layer of the skin. The most common type of squamous cell carcinoma develops in the stomach, lungs, and kidneys. Unlike these, cancer appears in tissues as sarcoma, leukemia, or lymphoma. While sarcomas originate from bone and soft tissue, leukemia originate from hematopoietic cells, lymphomas can originate from T and B lymphocytes from immune system cells, and multiple myelomas from plasma cells. As you can see, while there are many types of cancer, there are very few genetic mutations among them. These genetic mutations can either be activated by an oncogene or by turning off tumor suppressor genes that cause uncontrolled growth. Commonly mutated genes among different types of cancer include TP53, APC, BRCA1, BRCA2, and Rb, while commonly mutated oncogenes include mutations in growth-promoting pathways such as HER2, EGFR, RAS, and MYC.[21]

Current treatment protocols have options targeting the cancer cell to stop growth and progression depending on the tumor type. Common treatment strategies include removing the tumor by surgical resection, killing/stopping the growth of the cell with high-energy photons using radiation therapy, or using chemotherapy treatment with specific drugs aimed at interfering with DNA replication to kill the cancer cell. In targeted therapies, if a specific genetic mutation that causes cancer development is detected, depending on the type of cancer, the administration of drugs targeting only that mutation provides the treatment. This method is a very specific form of treatment only for a certain patient group. The genetic structure of the tumor is examined at the cellular and molecular level to determine the suitability of the patient to receive that treatment or not (eg: Administration of Trastuzumab for HER2 positive breast cancer and gastric cancer patients).[22] Trastuzumab is a monoclonal antibody that blocks the HER2 signal and keeps the cell in check and is administered as targeted therapy. Cell-based therapies such as immunotherapy and CAR-T (Chimeric antigen receptor T-cell) therapy are being tested as new treatment options.[23] Immunotherapy is a treatment modality that can mark cancer cells and modulate a patient's own immune system cells to fight cancer and make it easier to find and kill them. In CAR-T therapy, the patient's own immune cells (T cells) are extracted and the receptors on the extracted cells are changed to express the chimeric antigen receptor (CAR), which is known to attack cancer cells.[24] These altered cells are reintroduced to the patient where CAR-T cells go and find and kill dangerous cancer cells. CAR-T treatment is a new method, and certain cancers also received The United States Food and Drug Administration (FDA) approval for use in 2017.[25]

CONTRIBUTION OF CRISPR TO CANCER TREATMENT

CAR-T treatment potential can be improved with CRISPR technology. With this technology, the patient's own T cell receptors can be designed to express the CAR antigen, which recognizes the cancer cells of the patient who will receive CAR-T therapy.[24] Once designed in a lab environment, doctors can transplant the modified CAR-T cells back into the patient so that the new cells can attack the cancer cells. Applications on the regulation of T cells with CRISPR genome editing technology are in the testing phase. In experimental studies, CRISPR-edited CAR-T cells were found to be stronger against tumors.[26] CRISPR technology provides the power to change the software of cellular DNA. As new cancer mechanisms are defined, it is seen that cancer formation has a wide variety and different specific structures.[27]The importance of defining cancer according to its types and preparing a treatment protocol specific to it comes into play here. It is believed that CRISPR technology will contribute to the explanation of cancer biology. CRISPR allows for very specific and efficient editing of genetic sequences. For example; such as introducing specific site mutations, silencing or deficiency of genes of interest, altering gene expression or drastically altering the function of this gene, and adding large sequences. With CRISPR technology, cancers can be modeled more efficiently by editing certain genes in vitro. In this way, functional treatments and drug research can progress with larger-scale studies, more drugs can be screened at the same time, and the targets of these drugs can be defined faster.[28]

If we think of CRISPR technology as a CRISPR screen for oncogenes in cancer, it can be used to narrow the list of potential cancer-causing genes to identify new and relevant cancer targets. Although there are common mutations that open oncogenes and turn off tumor suppressor genes, the number of genetic control mechanisms that control the growth and spread of the tumor is low, and it is extremely difficult to completely eradicate without relapse.[29] Large-scale genetic or small molecule scans with CRISPR allow identifying new cancer-related genetic mutations or potential drug treatments. For example, on CRISPR genetic screens, barcoded cell libraries with genetic activation of cells can be prepared and the relevant tumor cells can be followed by injecting. The growth of these cells can then be monitored in vitro and the survivors can be recorded with the barcode method. The aim is that barcodes that survive over time provide a growth advantage over non-surviving barcodes and can indicate the level of oncogenic potential.[30]

CRISPR-Cas9 technology can be used to study the role of an oncologically determining gene. This technique has been used for the first time to understand how the inactivation of the BRG1 gene, which is commonly mutated in non-small cell lung cancer, contributes to the progression of cancer. At the end of the study, it was revealed that cells with BRG1 deficiency have increased replication stress and are sensitive to pharmacological inhibition of the ATR checkpoint protein, a potential therapeutic opportunity in the progressive prognosis of cancer and the treatment of lung cancer.[31]

Another application of CRISPR/Cas9 is that it can allow genetic screening that can be developed by deleting hundreds of genes individually to identify key regulatory proteins, potential drug targets, and/or test responses to experimental drugs relatively quickly. This approach can be modeled using a CRISPR/Cas9 genetic screen to identify potential therapeutic targets for lung cancer.[32] Deletion of histone, chaperone, nucleophosmin 1 by deleting more than 500 different genes individually that interact with or alter chromatin is known to significantly reduce tumor progression in vitro and in vivo. With this method, a potential tumor-specific drug target can be determined more easily.[32] Another important application of CRISPR/Cas9 screening allows the study of chemical-genetic interactions in how cancer responds to drug therapy. By screening with CRISPR, gene knockouts that act synergistically with the drug or therapeutic agent or confer resistance to the substance can be identified. In the first CRISPR scans, genome-scale knockout treatment of melanoma cells with BRAF inhibitor Vemurafenib was examined, and genes known to cause drug resistance were detected.[33] Similarly, when cancer cells were treated with TNF-related apoptosis-inducing ligand (TRAIL ligand) with CRISPR screening, it was able to reliably identify both positive and negative regulators of the TRAIL pathway.[33] As such, a series of CRISPR scans can be performed to identify the gene that modulates the cellular response to specific drugs such as ATR or Ras pathway inhibitors.

This strategy can also be used to investigate the mode of action of poorly characterized or unexplained anti-neoplastic drugs. For example,

apilimod is a known phosphatidylinositol-3-phosphate 5-kinase inhibitor, but the mechanism of its cytotoxic action is poorly understood. In CRISPR scanning, genes in the endosomal/lysosomal pathway were found to provide resistance upon knockout, and the cytotoxicity of apilimod was found to be critically based on endosomal/lysosomal homeostasis.[34]

Combined CRISPR screening approaches using lentiviral plasmids encoding two predefined single guide RNAs (sgRNA) have been designed by means of CRISPR technology.[35] Thus, by allowing dissection of the genetic interaction of binary gene deactivation, it also enabled the identification of synergistic drug targets. Han et al.[35] created a large pair of sgRNA libraries to identify 21,321 pairs of potential drug targets to test synergistic combinations in the Acute myeloid leukemia (AML) cell line. They have identified a strong synergy between MCL1 and BCL2L1 knockouts that can be reproduced using chemical inhibitors of the two genes. Similarly, Shen used the double inactivation screening approach to target the 73 cancer gene in duplicate, and a number of known (e.g. BRCA-PARP) and unknown synthetic killer interactions, some of which produced phenocopy using drug combinations.[36] There are many regulators of the Ras pathway, including EGFR as a known resistance gene. It is expected that CRISPR scans will be used to examine the chemical-genetic interactions of many more drugs, allowing an understanding of the contribution of tissue and genetic background to drug response. While most of the combined CRISPR screening techniques are designed to identify genes that show a particular phenotype, new screening approaches aim to gain a more general understanding of the biological changes associated with a particular gene confusion. As recently announced, combining single-cell RNA sequencing platforms with CRISPR screening will increase the explanatory power of CRISPR-based assays. Briefly, a pool of cells with different knockouts could be created, followed by single-cell capture and RNA sequencing of separate transcriptomes for each cell. An additional highly expressed guide barcode or marker can be added to the lentiviral vector to link the transcriptome of a cell with high confidence to the identity of the transduced sgRNA. These assays, called Perturb or CRISPR droplet sequencing (CROP-Seq), allow comparison of transcriptomes of cells with different gene knockouts, thereby grouping genes and allowing specific biological processes.[37,38] Using these methods, novel immune cell differentiation regulators and unknown aspects of the unfolded protein response in phenotypically homogeneous cell populations can be identified.

The genetic material in living things with a high organism's structure is DNA. After the information in DNA is transcribed into RNA, the function of RNA is to convert this code to protein. RNA consists of two components, one mRNA that is translated into protein and the other one non-coding-RNA (ncRNA) that cannot be translated into protein.[39] More than 98% of the RNA cannot be converted to protein and 70% of this nontranslatable part consists of introns. The number of non-coding RNAs that can only be identified in mammals today has exceeded 20,000. In cancer, the expression of non-coding RNAs is known to be unregulated, and also the transcription of oncogenes can be controlled by near and distant environmental elements. Therefore, a comprehensive understanding of non-coding elements will enable us to gain important information about cancer biology.[39] CRISPR/Cas9 technology is a powerful tool for querying non-coding items. Non-coding RNAs and gene silencing RNA interference (RNAi) are two different mechanisms that regulate gene expression. Silencers consist of several pathways that cause gene silencing and negative regulation of gene expression through small non-coding RNA fragments such as small interfering RNA (siRNA) and microRNA (miRNA). These non-coding RNA particles are responsible for the regulation of important biological processes such as development, proliferation, differentiation and apoptosis.[40] Abnormalities in miRNA function have been shown to play an important role in the etiopathology of cancer. Assessment of miRNA expression in cancerous tissues enables us to obtain information about the prognosis of cancer and thus the use of miRNAs as tumor markers. A biomarker for specific cancer types can be expressed with CRISPR technology. Revealing the miRNA-cancer relationship has started a new era in cancer research.[40,41] Changes in miRNA expression levels are associated with cancer development. The vast majority of target mRNAs negatively regulated by miRNAs are unknown. Hundreds of target genes of a single miRNA can be detected with bioinformatics approaches, but detailed methods to confirm this biologically are lacking.[41] It is necessary to determine the target genes in order to elucidate the biological and functional mechanisms of miRNA functions and to better understand their role in cancer. miRNAs have potential importance in cancer diagnosis, classification, and evaluation of prognosis. It will

be possible to make this determination with CRISPR technology. Since miRNAs, like transcription factors, act in different combinations in the regulation of several genes, it does not seem possible to use them in a therapeutic context. Thus, the administration of siRNAs that bind targets with higher specificity by different delivery methods make them more promising therapeutic agents for the treatment of cancer, human immunodeficiency virus infection, and neurological disorders in the future.[41,42] With CRISPR technology, we can identify siRNAs, use them as biomarkers, and even evaluate them as therapeutic agents in cancer treatment.[43] Another issue is that with CRISPR/Cas9 technology, modeling of organoids will become much easier. Organoids represent an excellent preclinical model for human tumors and are seen as an important field of study for the transition from basic cancer research to clinical practice. Organoids have been created to observe gastrointestinal development or disease pathogenesis in experimental studies. Fujii et al.[44] derived organoids of human gastrointestinal tissue and digestive system.[45] CRISPR/Cas9 genome editing technology has allowed it to present DNA double-strand breaks at specific genomic loci to edit or silence genes more efficiently than other technologies. Patterns of infection, inflammation, regeneration, and carcinogenesis can be developed in the tissues of the intestine, stomach, esophagus, liver, pancreas from mouse and human epithelial pluripotent stem cells. This 3-dimensional tissue can be tested indefinitely at different levels, and it can provide the ability to interpret cellular complexity according to its organs and systems.[44,45] CRISPR/Cas9 and organoid technologies have been combined to study the effects of certain genetic changes on tissue function and disease development. Fujii et al.[44] tried the use of organoids and CRISPR/Cas9 to examine non-neoplastic gastrointestinal diseases such as cystic fibrosis, multiple intestinal atresia, microvilli inclusion disease and Diacylglycerol O-Acyltransferase 1 (DGAT1) deficiency.[45,46] Patient-derived organoids have been used to study specific DNA methylation patterns in Barrett's esophagus and intestinal mucosa affected by Crohn's disease. Gastric organoids have been used to examine the effects of Helicobacter pylori infection.[44-46] These combined technologies can also be used in regenerative medicine studies. Researchers were able to inoculate human colon organoids on the surface of the mouse rectum and create a lineage tracking system for leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) positive colon cells.[47-49] A separate group of researchers demonstrated that human bile duct organoids can replace damaged mouse gallbladder walls or common bile ducts.[50,51] Genetically modified organoids are also ideal for drug screening and identification of genetic factors that affect drug activity and metabolism.

Another factor is Fusion Oncogenes (FO) and recurrent genomic findings in cancer. Fusion Oncogenes are characterized by patient-specific genomic breakpoints that occur in intronic regions and rarely disrupt coding sequences. Analysis of Cancer Genome Atlas data shows that FOs cause cancers to develop more than 16%.[52] Fusion Oncogenes, including mesodermal cancers (typically leukemia, lymphomas, and sarcomas) have also been found in epithelial cancers, including prostate, colorectal, breast, or melanoma, and to date contain many genes; More than 350 recurrent FO have been identified.[53,54] Given the limitations in cancer cells, FOs are attractive targets for directed therapy. Therapeutic targeting of FOs has remained challenging due to difficulties in specifically recognizing and targeting the resulting chimeric protein, as well as the need for an effective intracellular approach for delivery of therapeutic molecules targeting chimeric transcripts/proteins, as the FO products are intracellular.[53] Small molecules, intrabodies, and aptamers have been used successfully to target fusion proteins, antisense RNA, ribozymes and RNAi.[53] Similarly, the development of genome editing approaches offers new possibilities to directly target and modify the genomic sequence of cancer cells. Due to the cancer-causing role of FOs, their limitations on the cancer cell, and the dependence of tumor formation on these conditions, they are ideal therapeutic targets for the development of new targeted cancer treatments. Difficulty of working with drugs that target FO has an edible potential with CRISPR technolog.[53-55] Although some successful anti-cancer drugs have been developed based on the ability to target FOs, there is still a need for the development of new treatments. The high-throughput CRISPR/Cas9 genome editing pathway opens up new possibilities for the ability to precisely manipulate cancer cell genomes to correct or eliminate cancer-causing abnormalities and to develop FO-targeted options to eliminate cancer cells.

DISCUSSION AND CONCLUSION

Nowadays, especially in cancer treatments, it is very difficult to organize treatment protocols

according to the characteristic structure of the cancer cell, that is, the tissue/organ from which it originates, and the survival strategies it develops in the body. It is an acknowledged fact that the current treatment protocols alone are insufficient and there is a need for combined and more importantly, personalized treatments. Scientists involved have attempted to find a customized, effective, and easy way to correct changes that occur by manipulating DNA. Although several gene-editing methods have been developed over the years, none were fast, easy, inexpensive, and technologically suitable for widespread use. CRISPR technology has dramatically changed the line between the possible and the impossible and has thrown the research world into a different excitement. Currently, CRISPR studies are continuing in laboratories all over the world. CRISPR technology also promises many innovations in the field of oncology. Due to the convenience, simplicity, and affordability of the technique, it has become a mainstream methodology used in many cancer biology studies. CRISPR technology provides the power to change the structure of DNA today. Barcoded cell libraries with genetic activation of cells can be prepared on CRISPR genetic screens. By editing certain genes in vitro, cancers can be modeled more efficiently, and cancer development can be prevented over time. Functional treatments and drug studies can progress with larger studies, more drugs can be screened at the same time, and drug targets can be defined faster. With CRISPR technology, although there are common mutations that open oncogenes and turn off tumor suppressor genes, a genetic control mechanism that controls the growth and spread of the tumor can be provided. By developing genetically modified organoids, ideal environments will be provided for drug screening and identification of genetic factors affecting drug activity and metabolism. With CRISPR technology, one of the immunotherapeutic methods can also contribute to the potential curable effect of CAR-T treatment, which will greatly contribute to current treatments. Cancer treatment protocols will change drastically as CRISPR technology becomes widespread and studies yield results. Drugs will be targeted and will be studied mostly at the molecular level. Targeted and personalized treatments will replace today's conventional therapies.

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