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CRISPR-dCas9 in Cancer Epigenetic Therapy Progress

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Abstract:

Cancer appears and develops not only related to genes but also takes an important role in epigenetic regulation. In recent years, CRISPR-dCas9 as a new precision gene-editing tool has aroused much attention because it can combine with epigenetic effectors to regulate cancer-related genes specifically. It shows great prospects for treating a wide variety of diseases, particularly for epigenetic modifications of cancer. This article reviews the biochemistry of CRISPR-dCas9 systems and their use in epigenetic modification of cancer, focusing on how DNA methylation, histone modification, etc., affect tumorigenesis. In addition, the recent progress, challenges, and prospects of CRISPR -dcas9 mediated epigenetic editing technology for cancer gene therapy are also presented. By analyzing the current problems encountered in the clinical translation of CRISPR-dCas9 technology, this article has shown that CRISPR-dCas9 technology has the potential to become a targeted cancer treatment, which can also provide references for future medical research.

Keywords:-CRISPR-dCas9; Cancer; Epigenetics; gene editing; Tumor immunity

I. Introduction

Cancer's pathogenesis study is a complicated part that includes genetic and epigenetic elements. Recently, with the development of epigenetics, there has been a new way for us medical pros to look at it and realize how cancer occurs and develops, as well as why some people have a higher risk for certain cancers. Epigenetics mainly contains DNA methylation, histone modification, and non-coding RNA. These mechanisms are an important part of the tumor gene expression, a new way for treating the cancer and target. Take a look for example at how studies show that when DNA methylation is abnormal it has something to do with lots of different types of tumor development, particularly bad ones like Acute Myeloid Leukemia (AML) there seem to be some pretty big changes in how genes work because they're linked with stuff going wild up above them on proteins called Epigenetics [1].

Traditional (epigenetic drug has the problem of not being specific and having many side effects. This makes researchers want to find more exact regulation tools. CRISPR-dCas9 system belongs to the non-cutting gene editing technology. It can precisely regulate the specific site in the epigenetic state by combining it with various kinds of epigenetic regulators. The

advantages of this technology are that it can be flexible and efficient, and researchers can find out the regulation function of genes by using different cancer models, and then establish a customized plan. Furthermore, the application progress of the CRISPR-dCas9 system in cancer treatment has also attracted much attention. In research, they've found out that CRISPR-dCas9 could sufficiently control tumor gene expression which was a lot to improve the chemical treatment sensitivity of the tumor tissues [1]. But there are still lots of obstacles in current research, like off-aim effect, good transfection techniques, and how to take this from the lab to the doctor's office.

In summary, with more and more studies on cancer epigenetics, CRISPR-dCas9 technology is showing great promise as a novel regulator. It can not only bring healthcare professionals a new perspective to understand the complexity of cancer but also lay out a new avenue for cancer treatment in the future. Future research will focus on how to apply the technology in the clinical translation and to provide more treatment for the cancer patient as well as to improve the prognosis of the cancer patient's prognosis.

II. CRISPR-dCas9 biological principle

A. Basis of CRISPR-Cas9 system

CRISPR–Cas9 system from a bacterial immune defense mechanism, records information on CRISPR sequences of a viral DNA and then infects it again with a Cas9 nuclease along with single guide RNA (sgRNA) to target and cut the DNA. The Cas9 system's most important part is the Cas9 protein, it recognizes certain DNA through sgRNAs and makes double-strand breaks to edit the genome. CRISPR-Cas9 has very high efficiency and accuracy. It's a game changer in gene editing fields [2] Through the mutation of the nuclease activity of Cas9, dCas9 which is the form with no cleavage activity still can bind to the DNA and is capable of gene expression modification. After dCas9 binds to sgRNA, transcription factors or repressors could be recruited to accurately regulate target gene activities, thus offering a novel approach for gene function studies and manipulation [3] CRISPR – Cas9 has been widely used in plants and gene-editing in animal models of disease, cancer, and gene therapy. In agriculture. In this field, it has improved crop disease resistance and yield in precise editing of the genome of wheat and maize [4]; Challenges such as off-target effects and delivery problems will be improved. CRISPR-Cas9 will continue to innovate in the medical and agriculture fields [5].

B. dCas9 and Epigenetic Effector Fusion Strategy

dCas9, and epigenetic effects (DNMT3A, TET1, EZH2, HDAC1) can be accomplished without having the DNA cutting precision of epigenetic regulations: sgRNA-guided dCas9-effector complex targets gene promoter or regulatory element like dCas9-VP64 activates POU5F1 and SOX2[6]. Also for cancer treatment, it can use dCas9-DNMT3A or TET1 and this will change the methylation within the cancer cell and reverse abnormal methylation [7]. Dcas9 could also use the engineering design to create a flexible multi-function regulating system via the combination with effector p300 (enhancing gene expression) or KRAB (reducing gene expression)

C. Advantages of CRISPR-dCas9 Epigenetic Editing and Limitations of CRISPR-Dcas9 Epigenetic Editing

The biggest benefits of crisper dCas9 are highly specific, reversible, and no genetic damage. And it can also accurately control cancer's epigenetic state, and will not lead to serious DNA damage, which is beneficial to the recovery of normal gene expression [7]. Limitations include off-aim, delivery, and immunity. Design optimization can reduce the occurrence of off-target, but it will still cause abnormal gene expression. Delivery systems struggle with inefficient cell membrane penetration, limiting therapy potential Moreover, extrinsic CRISPR materials may also trigger immune reactions, affecting intracellular applications.

III. The mechanism of epigenetic modification in cancer development

A. Abnormal DNA Methylation

DNA methylation falls into some important epigenetic processes that concern gene expression and abnormal conditions become critical factors regarding tumor development. Hypermethylation in tumor suppressor gene promoter causes silence of the gene and leads to tumorigenesis. DNA methyltransferases (DNMTs): The core function is in this process, DNMT1, DNA DNMT3A/B in maintaining methylation, and new methylation. The research found that abnormal DNA methylation was very often an early event, and it was tissue-specific. For example, from the genome-wide methylation levels, there is a vast increase in the patients that got bladder cancer which means that this is a potential early indication [8]. On the contrary, hypomethylation of oncogene promoters causes it to activate its expression, enhance cell propagation, inhibit cell death, and expedite tumorous development [9]. In ad-

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dition, it affects gene expression variation via controlling m RNA polyadenylation and this is related to cancer treatment response and outlook [10]. Methylation analysis technology from high-throughput sequencing sulfite process development greatly improved the genome-wide methylation maps analytical accuracy, cancer development mechanism, and treatment strategy development laid the foundation. For instance, DNA methyltransferase inhibitors reverse methylation and stop tumor development. Studying the DNA methylation patterns will give us a new way for early diagnosis and individual cancer treatments.

B. Abnormal Histone Modification

Histone alterations control gene expression through changes in chromatin structure and transcription. Acetylation is usually chromatin relaxing and gene actuation, deacetylation is usually transcription inhibition. Methylation can be either activating or inhibiting the effect depending on the location of modification and regulators [11]. And these kinds of imbalances in cancer will push it forward. An example is HDAC overexpression silencing tumor suppressor genes [12]. Histone marks interact with non–coding RNAs in digestive system tumors where it can change cell growth, movement, and blood vessel growth. Regarding histone modification of targeted therapy drugs (e.g., HDAC inhibitors), there have been promising clinical outcomes in reversing a modified tumor phenotype back to normal [13]. To have a better understanding of how histone modification dynamically changes would be good for us to develop more precise cancer treatments.

C. Epigenetic Regulations on Non-coding RNAs

Noncoding RNA (ncRNA) is important for tumor epigenetic regulation, mainly including miRNA and lncRNA. To participate in the development of tumors through the regulation of the expression of epigenetic enzymes as well as to show significant regulatory functions in ensuring cellular function and tumorigenesis. In breast cancer (BCa), the expression of ncRNAs is closely associated with tumor initiation and progression. These ncRNAs regulate the expression of epigenetic enzymes, impact the signaling pathways in the tumor microenvironment (TME), and support the tumor's progress. In the research of MI, miRNA regulates myocardial cell inflammation, apoptosis, and regeneration and influences cardiac remodeling and functional recovery, outside secret body ncRNA via cell-to-cell signal transmission, having prospects for CV diagnosis and treatment. In colorectal cancer, lncRNAs regulate the expression of target genes by interacting with miRNAs and influence the occurrence and development of tumors. Clearing up the regulatory rules of ncRNA would give the grounds for creating treatment plans and figuring out tumor diagnosis markers for the very early stage. NcRNA modulating epigenetic enzymes affect tumor-associated biological processes, it brings new ideas about the molecular mechanism and clinical treatment.

D. Epigenetic Regulation of Tumor Immune Interaction Microenvironment

Epigenetic regulation, mainly through DNA methylation and histone modifications, influences the TME by affecting gene expression and contributing to immune evasion. It involves not only tumor cells but also the regulation of immune and stromal cell functions within the surrounding microenvironment. BCa, from a survey of around 400 different kinds of epigenetic regulation factors can form a functional module, and a lot of factors inhibit the immune gene expression to promote the escape of tumor immunity. Epigenetic changes may also affect the maturity and function of NK cells, thereby hindering their battle against tumors. And epigenetics regulation can be reversed, it's opened a new way to treat disease. Using epigenetic inhibitors can revert the abnormal tumor cells, it will return the immune cells to their normal function, then the immune cells start to play the anti-tumor role. It is also showing some promise in pre-clinical [14]. Epigenetics interacts with the tumor's immune microenvironment research finds out the escape of immunity, giving a theoretical premise to target therapy. To increase the effects of immunotherapy through controlling epigenetic mechanisms, it is necessary to conduct more research on this matter.

IV. CRISPR-dCas9 mediated DNA-methylation-modification used in cancer therapy

A. dCas9-DNMT3A-Mediated Gene Methylation

Based on CRISPR dCas9 – DNMT3A system can do particular DNA methylation and gene silencing. Aim for the tumor-related-gene promoters results in large-scale cancer cell proliferation inhibition due to methylation. Like KRAS gene promoter methylation regulating the expression of gene and inhibit cancer cells growing. dCas9 is inactive Cas9, and it can accurately target methylation by combing DNMT3A. There's been some studies showing that MGMT promoter methylation in cancerous glioma cells can bring on reducing MGMT expression and sensitizing to Temozolomid. In addition, methylation of PLA genes in BCa cells greatly reduced its expression and the proliferation and invasion of the cells [15]; dCas9-DN-

MT3A changes the biological behaviour of tumors by regulating their epigenetic status, it gives us the strategy on targeting other kinds of oncogenes.

B. dCas9-TET1-mediated Gene Demethylation

The dCas9-TET1 system restores tumor suppressor gene expression by targeting DNA demethylation. For example, in the case of FOXP3, hypermethylation can silence the gene, impairing its ability to suppress BCa, thereby allowing tumor cells to proliferate unchecked. This system fuses the catalytic domain of TET1 to dCas9, enabling locus-specific demethylation of targeted genomic regions. For instance, demethylation of the TRIM58 gene in clear cell renal cell carcinoma (ccRCC) has been shown to promote transcription and inhibit tumor progression [16]. Additionally, VP64, when combined with other transcriptional activators, enhances gene reactivation and improves the therapeutic potential of this approach [17].

C. Methylation Editing Improvement

The SunTag system can improve the efficiency of methyl editing by using multiplex-effectors-complex. Targeted recruitment of TET enzymes or DNMT3A would be able to efficiently regulate DNA methylation status, providing a potential tool for understanding the epigenetic mechanisms of disease. Combining it with the KraB-mecP2 gene silencing strategy, which can combine the transcriptional inhibition of KRAB and the binding of MeCP2 to methylated DNA, so as to increase the specific target gene silencing effect and reduce off-site effects. Optimization strategies make it more precise when editing methylation and set up the technical framework for gene regulation in cancer treatments and various areas.

V. CRISPR-dCas9 mediated histone modification regulation and its role on cancer therapy

A. dCas9-histone Acetyltransferases (Such as P300) Promote Gene Activation

Histone acetylation regulates genes by modifying chromatin. Use the dCas9 combined with histone acetyl transferase p300 to form a complex that targets the specific gene promoter region, increases the degree of histone acetylation and activates the target gene expression. As a transcription coactivator, it helps with chromatin remodeling and phosphorylation of RNA polymerase II through acetylation, so as to improve transcription efficiency. Take colorectal cancer as an example, activation of tumor suppressor gene ZNF334 by dCas9-p300 results in suppression of tumor growth through regulation of cell cycle and induction of apoptosis [18].

B. Dcas9-histone Deacetylases Like HDAC1 Meditate Gene Silence

Dcas9 binds to Hdac1, and so, when fused it targets onco gene promoters and transcription is repressed by deacetylation. An example is that when dCas9-HDAC1 complex goes to the KRAS gene, it will reduce the gene very much and then stop the growth of the cancer cell which leads to cell death; Compared with conventional therapies, it's much more precise and it would reduce the effect on normal genes. By altering the design of gRNA, it is possible to silence a wide variety of oncogenes and provide the possibility of personalized therapy. In the future technical optimization will allow for its use in the clinic.

C. Histone Modifications Editing Challenges and Prospects

Currently histone modification editing is still plagued by low delivery efficiency and poor regulation specificity. Dynamic histone modification is related to cell state and environmental conditions. Also, it might be better for many histone modifications, like H3K4me3 and H3K27me3, as these could boost control over gene expression. Combining them with machine learning for predicting modification patterns also makes the regulators more effective [19]. Histone modification editing still faces many problems, but it is very promising in the field of cancer, metabolic diseases, etc. Future research will further comprehend this, and it is expected to be used clinically.

VI. CRISPR-dCas9 on regulating TME

A. Epigenetic Regulations on Immuno-suppressive Microenvironment

Formation of tumor immune suppression environment is related to epigenetics regulation It was found out that to silence HIF1α gene by CRISPR-dCas9 – EZH2 system can reprogram tumor- associated macrophage and enhance antitumor immunity. HIF1a being a transcription factor activated under a hypoxia condition leads to promote tumor metastasis and invasion with high levels of overexpression and forms a state of an immunosuppressed environment when in the TME. This system could be used, and also lower the production of immunosuppressive factor, the function of tumor infiltration T cell could be improved, thus the immunotherapy effect could be enhanced. And also, Hif1a joins forces together with MDSCs, better

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identified as bone marrow suppressor cells, and macrophages to manage getting away from the body's immune systems, this is because Hif1a influences cytokines linked to immunosuppression, which means it shows possible in combination therapies focused around its downstream actions.

B. Epigenetic Control of Immune Checkpoint Genes

Epigenetic modification of immune checkpoint genes such as PD-1, CTLA-4, etc. plays an important role in the expression and tumor immune escape. CRISPR-dCas9 tech can control these genes by targeting DNA and histone changes. For example, the DNA methylation of PD - 1 / CTLA - 4 is associated with the expression level of mRNA and response to immune therapy, while different methylation signatures serve as predictive biomarkers. Design the gRNA, let CRISPR-dCas9 target specific immune checkpoint genes, enhance checkpoint inhibitors' efficiency, diminish the bad impacts of these inhibitors and give fresh hope to patient-specific treatment

C. CRISPR-dCas9 +Immunotherapy Combination Strategy

CRISPR-dCas9 system can guide dCas9 protein to the target gene area through sgRNA, so as to realize transcription regulation without changing the genome structure. Combined with immune checkpoint inhibitors, it can significantly enhance the function of effector T cells and antitumor immunity through activation of tumor antigen expression, inhibition of immunosuppressive factors such as PD-L1[17]. For example, combined application can reverse the immunosuppressive state of the TME, reduce drug resistance and improve survival [20]. But the technology's delivering system has to be improved too, so that off -target effects can be lowered, and its use in patients becomes possible.

VII. CRISPR-dCas9 technology application examples in various types of cancer

A. BCa

BCa is a kind of high morbidity and mortality female malignant tumor, its occurrence and development are closely related to the regulation of PLAU gene expression. PLAU encoded enzymes have a big impact on how BCa spreads because they make tumor cells move and invade, and PLAU is linked to things like how advanced it is and what kind of problems it causes, so it could be useful for treating it. CRISPR-dCas9 can inhibit the formation of BCa cells through activating the FOXP3gene and it could increase the ability of immune cells to eliminate cancerous cells by adjusting the TME, this gives rise to a new strategy for gene therapy. And, as a good carrier of CRISPR/Cas9 gold nanomaterials, which increases genetic editing efficiency and targeting due to their biological compatibility; gold nanomaterials are the basic of precise therapy. At present, researchers use regulations of PLAU, CRISPR technology and nano delivery systems to further develop and advance various kinds of accurate and effective BCa treatments.

B. Colorectal Cancer

The dysregulation of epigenetic regulators such as ZNF334 contributes to the development of colorectal cancer. ZNF334 activates tumor suppressor gene expression via histone acetylation, which inhibits tumor progression and is a new target to overcome tumor drug resistance. CRISPR/Cas9 -Based epigenetic editing technology can inhibit the proliferation and migration of cancer cells by re-expressing tumor suppressor genes, and it has promising therapeutic applications [21].

C. Other Cancers

ZAR1's epigenetic silencing is discovered in several cancers. Zar1 turning on again could return tumor-suppressing functions, through un-doing DNA methylation or histone modifications, and this might be a new thing for fighting many kinds of cancers. KRAS gene is abnormally activated so as to suppress tumor by means of RNA interference or small molecule drug silence. it also true on pancreatic cancer and colorectal cancer, the silencing effect that KRAS gene produce works together, TME and immune response, to personalize treatment [22]. right now, it's looking into a combo of epigenetic intervention and targeted, immunotherapy right now, and giving some ideas to improve life expectancy and quality of life for people with cancer

VIII. one of the most recent technology on CRISPR -dCas9 epigenetic editing

A. Multifunctional Fusion Protein Design

Multifunction fusion protein design has great potential in the field of cancer treatment and gene therapy. Using the transcriptional activatior repressor and different epigenetic enzymes that needs to be controlled the function of the cells on different layers and this way create more potent and specific therapy. Take an example, the researchers

have generated a fusion protein DNA conjugate with genome degrading peptide and mitochondrial specific pro-apoptotic peptides synergized to inhibit both drug sensitive and drug resistant tumor cells, and optimized the drug stability and targeting as well as decreased the side effects on normal cells. Genetic engineer technology is also capable of building a selection drug delivery system to improve the targeted delivery rate of anti-tumor drugs. In another study, a composite protein containing an anti-IL-1RAP SCFV, a cell-penetrating peptide and an endosome lytic peptide was built to and was successfully delivered to human myeloid leukemia cells; it was proved that the constructed protein showed efficient cellular uptake and endosome lytic activity in vitro. In addition, genes fusion of elastin like peptides and ordered structures can get stimuli responsive composites, which could realize the precise manipulations of cell behaviors by regulatory the structure, and also promote the development of biomaterials and cell engineering. Advances are being made to offer new solutions for precision medicine and showing that multifunctional fusion proteins are useful in clinic.

B. Multi-gene co-regulation by Dual CRISPR

Using dCas9 and sgRNA from different origins will have the dual CRISPR to control the multiple genes expressions at once, improving the flexibility of gene editing For example, by using different dCas9s to work together to turn on or down target genes at the same time, researchers can control plant growth and development more easily than through gene editing with just one gene at a time (for example) [9]. In cancer it can also be applied to study the gene interaction network and the pathological mechanism and offer a new way to study these complicated illness. But at the same time, it also be needed to optimize the system such as screening effective dCas9 variants, designing exact SgRNAs with low off-rate, and enhancing the delivery efficiency in vivo using Nano technology [12]. With the advancement of technology, the double CRISPR system will be used even more in functional genomics and precision medicine.

C. Use Instant Back-revision and High-Throughput Screening to Improve Editing Efficiency.

Real time feedback has combined with HTS and this has made gene editing more efficient. Single-cell sequencing could be used for analyzing the different types of edited cells, for example, real-time monitoring of the gene-editing result on different cellular subset via single cell sequencing RNA to guide experiment [23]. Epigenetics + HTS (high throughput screening) = able to screen for chemicals that can regulate the state of epigenetics, like using compound libraries to look for auxiliary instruments to activate autophagy for an increase in gene target expression. Automated process of HTS greatly reduces the length of the experimental cycle and lowers research costs. It's fast data output speeds up the improvement of editorial strategies and promotes the development of precision medicine. Joining this tech brings a new flavor to the gene editing side of the board and has a different way of handling really hard biological problems

IX. Challenges and prospects for CRISPR-dCas9 gene therapy on epigenetics:

A. Off - target Effect and Control Strategy

Off-target effect is the most important problem caused by unexpected mutations of non-target DNA sequence editing which is applied in CRISPR/cas9. To some extent, this can greatly reduce the probability of off-target by reasonably optimizing the base composition and length of sgRNA, then carrying out computational simulation to optimize the sgRNAs and use high-throughput screening. There are studies showing that Cas12a variant with strict PAM recognition characteristics could both reduce off-target effects and keep high editing efficiency. dCas9 novel version can also be designed to bind to target with higher specificity, for instance, the dCas9-DNMT3A-DNMT3L system is able to place DNA methylation at a specific location on DNA and lower non-specific binding. Machine learning and computational biology is driving the development of a better off target prediction. DeepCas13: The model successfully applied deep learning for predicting Cas13d targeting and off-targeting effects. And combined with the development of highly specific sgRNA, the development of dCas9, and artificial intelligence technology to improve the safety and application perspectives of genome editing.

B. Personalized Precision Medicine

An EPIC gene editing treatment plan according to the patient's epigenetic characteristics will greatly improve the accuracy of the treatment, especially in the treatment of the tumor, such as acute myeloid leukemia. It is very useful to consider the genetic background and the molecular characteristics to develop a strategy. Big data, bioinformatics push precision medicine, it locates disease markers with multi-omics analysis to raise efficiency, decrease side effects. Patient derived organoids combined with a microfluidic chip is a new in vivo treatment testing platform

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[24].

X. Conclusions

Gene editing tech develops really fast, so CRISPR-dCas9, this super precise type of editing that affects the way genes are used but not the actual DNA letters, is giving new chances for treating cancer. The CRISPR-dCas9 has been applied in therapy and this article reviews the applications of it in cancer therapy including breakthroughs in the treatment in DNA methylation targeting, histone modifications and modulating the TME of cancer cells. These apps enhance the efficiency of regulating cancer-related genes and have great chances of treatment, which is a big change in how cancer is handled.

However, although it is exciting with the CRISPR-dCas9 technology. Firstly, delivery efficiency is the biggest factor of successful gene editing, there aren't yet univeral methods for delivering CRIPSRs that can do both efficiently and specifically. Secondly, off-target effects, there are still some hidden dangers from gene-editing technology that might trigger an unanticipated modification on the non-targeted genes, causing a bad reaction. And pay attention to the possible immune response caused by CRISPR-dCas9, which will not only affect the curative effect, but also bring a series of negative effects to the health of the patient. As science and technology progress, and people delve deeper into clinical application, CRISPR - dCas9 will become progressively significant for personal cancer therapy. CRISPR-dCas9, together with top-notch delivery systems and correct methods to regulate gene action, can not only let sick people have special treatment options, but maybe also make treatments much more successful and help patients feel much better. CRISPR-dCas9 tech's quick development gives a new path for treating cancer. Despite facing many problems, it cannot be denied that it has some value clinically.

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