CRISPR-Mediated Demethylation Reactivates Tumor Suppressor Genes in Cancer Cells

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DNA Methylation is a body's natural ability to add or remove methyl groups to DNA in chromatin to prevent or enhance gene expression of that gene. Here, this study explores the use of CRISPR, a modern gene editing technology, to help demethylate or methylate certain genes to reverse epigenetic silencing. This study aims to identify possible areas of further research in epigenetic modifications using CRISPR. Different topics regarding gene regulation and cancer and various ways CRISPR has been utilized were researched using research tools such as PubMed and Google Scholar. Several papers have used CRISPR for DNA methylation and gene expression. This opens up possibilities for researchers in labs to work with CRISPR as a gene editing tool to target specific known cancer-causing regions of mutated genes. Many papers have emphasized the significance of using Cas9 fused proteins for better efficiency and accuracy for specific epigenetic modifications. However, further research should be conducted because fusion proteins are a relatively new area of cancer research, and CRISPR comes with many implications, such as off-target effects that could be harmful.

Keywords: DNA methylation, Hyper methylation, Hypomethylation, CRISPR, Cancer, Oncogenes, Tumor Suppressors, Epigenetic Modifications

Introduction

Cancer is a disease where cells grow uncontrollably, which is a result of mutations or alterations in the DNA. Cancerous cells are abnormal because they proliferate, and are abnormal in shape/size or number of nuclei. This can cause large tumors that are harmful to one's organs. Tumors are a group of cancerous cells that can affect the function of nearby organs. The hallmarks of cancer display the capabilities required for a cell to progress from normal to cancerous (Fig.1). They comprise six capabilities that a tumor acquires to thrive in the microenvironment. The first hallmark of cancer is the ability of a tumor to sustain proliferative signaling. In normal cells, the semi-permeable cell membrane can regulate the production and release of growth-promoting signals. However, cancerous cells can produce an excess amount of these signals to increase growth. Another hallmark of cancer is the cancer cell's ability to evade growth suppressors. In healthy cells, mechanisms like tumor suppressors, limit cell growth and proliferation; however, in cancerous cells, tumor suppressors are evaded through genetic and epigenetic mechanisms. The third hallmark of Cancer is the cell's ability to resist cell death. Normally, cells have a mechanism to induce apoptosis, or controlled cell death, in extreme cases where the cell is harmful. However, tumors increase the expression of anti-apoptotic regulators, which helps maintain the cell's survival. The fourth hallmark of cancer is enabling replicative immortality, which represents the cancer cell's need to continue to replicate to become a macro tumor. In normal cells, the telomeres, the protective ends of our chromosomes that prevent their degradation, will shorten, and the cell will reach a point where cells will no longer replicate. However, cancerous cells activate an enzyme called telomerase, which can preserve the telomere and allow for unlimited replication of cells. Additionally, the fifth hallmark of Cancer is inducing angiogenesis, where the tumor releases growth factors that function as connective tissue from the blood vessels, providing the tumor with the nutrients and oxygen it needs to thrive. Finally, the last hallmark of cancer is activating invasion and metastasis, which is a multi-step process where tumors can break off and travel through the blood or lymph system to colonize other parts of the body, which can be critical. Two main genes are involved in the tumorigenesis of cells, where normal cells develop into cancerous cells: oncogenes and tumor suppressors.

As mentioned above, for the purpose of this review, the focus will be on tumor suppressors and oncogenes and their significance on the tumor microenvironment. Proto-oncogenes and tumor suppressors are normally regulated, but their function is manipulated or lost when they are mutated. Tumor suppressors normally regulate cell division, among many other functions like repairing DNA or inducing apoptosis. Some tumor suppressor genes include p53, which functions in apoptosis, VHL, which functions in cell death, and TGF-beta, which acts as an antipro-

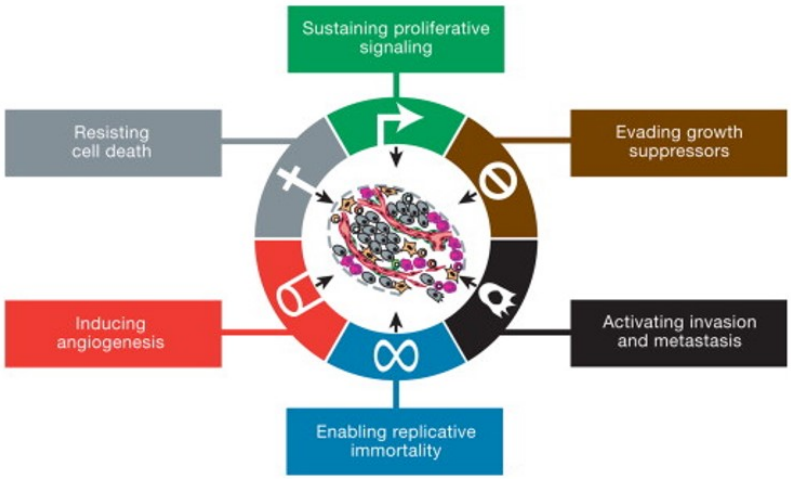

Fig. 1 Hallmarks of Cancer (Douglas Hanahan, 2011)

liferative gene. It is important to note that these are not the only functions of these genes. For example, TGF-beta also functions in the activation of immune cells, anti-inflammatory effects, and preservation of bone health as well. Proto-oncogenes, simultaneously, function in promoting cell growth and division, along with many other cellular processes and growth factor regulation. An example of a proto-oncogene is the HER2 protein, which aids in cell growth and division of breast cells. While tumor suppressors and proto-oncogenes are important for the maintenance of an organism's health, they can also prove to be harmful during tumor progression. Ultimately, tumor suppressors can be inactivated and lose their function and proto-oncogenes can be overexpressed and become oncogenes, leading to uncontrolled cell growth in both cases. This overexpression or inactivation can result from genetic and epigenetic alterations, specifically hyper or hypo methylation causing tumor initiation and progression.

Some of these genetic alterations that are caused by differences in gene expression in the DNA are due to hypermethylation or hypomethylation of DNA. DNA methylation is an epigenetic alteration or modification that involves the addition of a methyl group(-CH3) to the DNA (Fig. 2). DNA methylation plays an important role in influencing whether a gene is expressed or silenced. DNA methylation in the silencing of genes is a process where a methyl group is added to a specific site on the DNA to block the proteins that typically help with gene expression. Another example of epigenetic modifications that can alter gene expression is histone modification through the demethylation or methylation of histones. This involves adding or removing a methyl group to specific amino acids on histone proteins. Histones are a group of proteins found in the nucleus of a cell and play an important role in the organization and packaging of DNA. They form chromatins by wrapping the DNA around them. In this paper, the focus will be on DNA hyper and hypo methylation and its effects.

DNA hypermethylation is caused by an excess amount of methyl groups added to the CpG islands in DNA. CPG islands are areas in the DNA with a high frequency of cytosine and guanine nucleotide bases. These islands are frequently found in the promoter regions or transcription sites of genes. Transcription sites are precise locations in the DNA, which marks where transcription begins for gene expression and protein synthesis. Promoter regions are specific sequences near the beginning of a gene, which regulate the initiation of transcription. CpG islands also have a high sensitivity to methylation. Therefore, normal and healthy cells have un-methylated CpG islands. Hence, the methylation status of CpG islands can influence gene expression. Methylated CpG islands can signal for proteins called methylbinging domain proteins and chromatin remodeling complexes. This can modify the structure of chromatins, making them more condensed and tightly packed. When DNA is hypermethylated, it inhibits the binding of RNA polymerase to the promoter regions. This can cause challenges for the gene to be expressed, which results in gene silencing. This inhibits access to the DNA, which silences genes and causes the loss of function. On the other hand, DNA hypomethylation is caused by the loss or reduction of methyl groups from the cytosine bases in DNA, which can lead to increased gene expression. When CpG islands are hypomethylated, it becomes easier for RNA polymerase to access transcription sites. Hypomethylation causes the chromatins to be less tightly packed, allowing more RNA polymerase to bind and initiate gene expression. This can often lead to the expression of unwanted genes that were previously silenced or the overexpression of genes. One important enzyme involved in the methylation of DNA is DNA methyltransferases (DNMTs), which include DNMT1, DNMT3A, and DNMT3B. DNMT1 is

responsible for preserving and copying the DNA patterns during DNA replication processes. However, DNMT3A and DNMT3B help in creating new methylation patterns. The normal function of DNMTs allows for stability in genes, regulating gene expression, and silencing repetitive parts of the DNA. However, when dysregulated, DNMTs can serve a vital role in the progression of cancer.

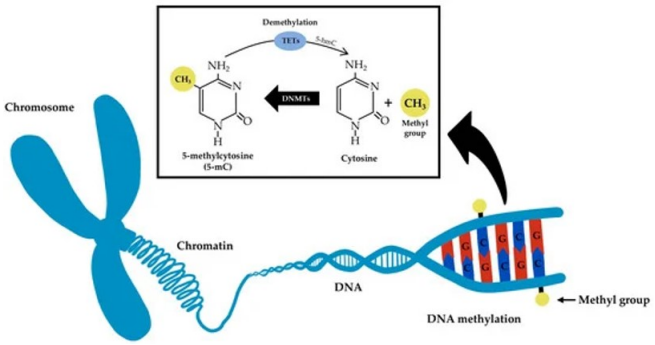

Fig. 2 DNA methylation process (Ana Valente, 2023)

The epigenetic changes mentioned above can be a key factor in the tumorigenesis of oncogenes and tumor suppressors. For example, tumor cells can initiate hypo methylation, which can increase the production of a beneficial gene for a cell to acquire tumorigenic capabilities. They can also cause proto-oncogenes to become oncogenes through tumorigenesis when healthy cells or proto-oncogenes are overexpressed, which causes a change in function, leading to abnormal cell growth. Similarly, tumors can activate hypermethylation in tumor suppressors, which can silence their function. This can lead to the body's inability to fight off cancerous cells. Both hypermethylation and hypomethylation can lead to the acquisition of various hallmarks in cancer, including sustained proliferation, evasion of suppressors, and resistance to cell growth and invasion.

Epigenomic editing can be an important step for reversing abnormal epigenetic modifications. Epigenomic editing in cancer is the process of targeted modification of epigenetic modifications in the DNA rather than the alteration of the actual DNA sequence. These modifications can be made through a gene editing tool called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). A subtype of CRISPR that is commonly used for making edits in the DNA is CRISPR/Cas9. CRISPR-Cas9 consists of three main components: DNA repetitive sequences, the Cas9 protein, and a guide RNA (Fig. 3). The guide RNA is a set of RNA bases that are complementary to the target sequence on the DNA. The guide RNA targets a specific location on the DNA and binds to it. Cas9 is an enzyme that binds to the DNA and makes precise cuts or a double-strand break. A double-strand break is a break or cut in both strands of the DNA. The Cas9 cleaves the area of interest and causes parts of the DNA to be broken off since it's exposed to other proteins and fluid in the nucleus. Then, the DNA is repaired through the cell's natural repair systems or a DNA template. The natural repair systems of the cells are non-homologous end-joining and homologous recombination. The non-homologous end joining joins the ends of both sides of the DNA. This can result in deletions or insertions that can disrupt the function. Homologous recombination is when the cell looks for the precise bases from the sister chromatid and recreates the area with a double-strand break. However, when using a DNA template, scientists can replace the original part of the DNA with a different sequence by using it as a template. The CRISPR cas9 system will proceed to use that DNA sequence with homologous recombination. In the case of epigenetic modifications, CRISPR will target specific Epigenetic marks. Epigenetic marks are the areas of the DNA that have epigenetic changes. Then, CRISPR can be used to add or remove methyl groups to the area of interest, which can help regulate the gene expression.

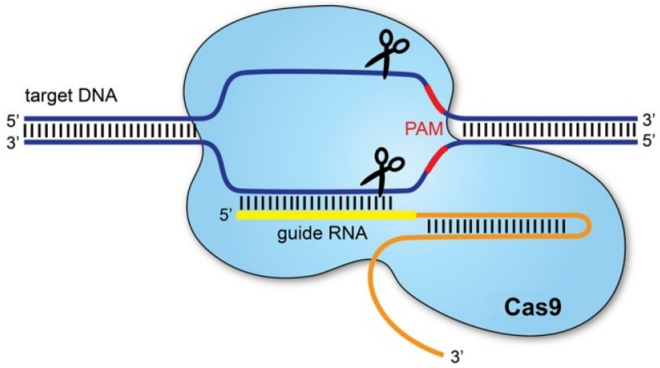

Fig. 3 Model of CRISPR/Cas9 system (Melody Redman, 2016)

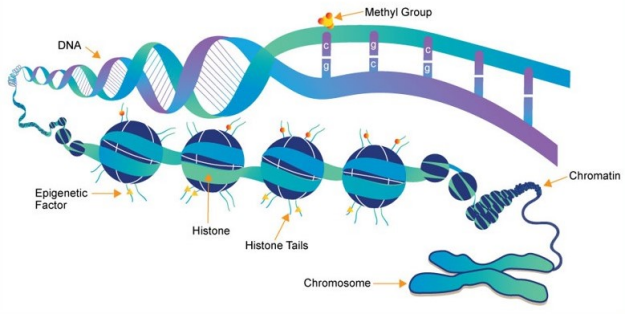

Fig. 4 Model of Epigenetic Marks (Judith Millan, 2020)

Methods

First, a list was made of all possible keywords by breaking up the research topic and introduction. Then, databases like Google Scholar and PubMed were utilized by entering keywords like "DNA methylation and CRISPR," "CRISPR and cancer," or "gene regulation and CRISPR". Furthermore, an outline was used to break down and plan the paper. Then, about 120 papers were reviewed to identify which would assist the paper by reviewing the abstract, methods, results, and conclusion. About 60 were extracted and proved useful as shown in Fig 5.

Results

Multiple papers can be seen where the use of CRISPR for different gene regulation techniques for cancer genes is described.

A study conducted by James Mcdonals in 2016[1] investigated the role of DNA methylation in the regulation of tumor suppressors and developed ways to reverse these changes. The paper discusses how the researchers used a protein called DNA methyltransferase (DNM3TA) and Cas9, which were linked to create dCas9-D3A. The researchers selected guide RNA that would allow for the least amount of errors and was an area of interest. They then used a virus to introduce the CRISPR system into human and mouse cells. The paper explored the connection between methylation-induced silencing of tumor suppressor genes and an increase in cell proliferation. They found a modified CRISPR/CAs9 system that was used to target and demethylate specific gene promoters in cancer cells. This approach was successfully found to reverse the epigenetic silencing of tumor suppressor genes, which led to their re-activation. The paper also suggests that reversing epigenetic silencing in this way can inhibit tumor growth and restore the function of tumor suppressors. This paper is important because it establishes a successful method of CRISPR/Cas9 and DNMT3A to restore the normal function of tumor suppressors through de-methylation of target areas. Since the use of CRISPR/Cas9 and DNMT3A was successful, future research can look at the viability and accuracy of using DNMT3A to assist in the de-methylation.

Another paper written by Chi Xu in 2022 explored how nicotinamide nucleotide transhydrogenase (NNT), a vital gene in Lung cancer, affects cisplatin (a chemotherapy drug) resistance and the role of DNA hypermethylation (Chi Xu, 2022)[2]. First, the researchers conducted an experiment that investigated the role that NNT played in NAD+ levels, autophagy, and the level of sensitivity of lung cancer cells to the drug cisplatin. They also examined the possibility that DNA hypermethylation can cause the dysregulation of NNT during the progress of cisplatin resistance. The study found that NNT plays an important role in reducing cisplatin resistance in lung cancer cells by reducing NAD+ levels, suppressing autophagy, and increasing sensitivity levels to cisplatin. This emphasizes the importance of NNT in preventing the growth of cancer cells. They also discovered that DNA hypermethylation could be responsible for the dysregulation of NNT. Thus, in the paper, the researchers demonstrated that modifying the DNA methylation status of NNT could help prevent cisplatin resistance. This is crucial because it shows how CRISPR technology can help reverse DNA hypermethylation of an important gene in lung cancer. This can be further tested by using CRISPR to help demethylate and measuring NAD+ levels to see if the method was able to affect the role of NNT.

In another paper, in 2020, Chang K. Sung aimed to investigate the potential of a modified CRISPR-Cas9 system to change DNA methylation patterns in order to reregulate gene expression (Chang K. Sung, 2020)[3]. The researchers focused on genes with altered methylation patterns associated with cancer. They first fused Cas9 nuclease with various enzymes, one of which was DNMT3A. The guide RNA was then used to guide the dCas9 (the fusion protein) to the target gene promoter. They conducted experiments in vitro, cell cultures, and in vivo in mouse zygotes. The paper found that the dCas9- DNMT3A could be used to methylate specific areas of the DNA, like gene promoter regions, which could successfully silence genes. The paper also explored more dCas9 fusion proteins, such as dCas9-Ezh2 and dCas9-KRAB, which proved to be efficient in gene suppression in their experiments. The paper successfully applied the dCas9 mechanisms for targeted gene suppression, as well as gene activation. Therefore, this paper shows how fusion Cas9 proteins can be successfully incorporated into the demethylation of oncogenes as well as the reactivation of tumor suppressor genes. By providing another example of the successful use of DNMT3A with CRISPR, this method is supported as efficient. The paper also offers other possible fusion proteins to look into and research using in vitro and in vivo methods.

Another paper further discusses this idea with the goal of exploring the potential of single guide RNA-directed catalytically inactive Cas9 with epigenetic effectors (sgRNA-dCas9-EE) as a tool for targeting epigenomic modification in association with cancer (Mohammad Mijanur Rahman, 2020)[4]. The researchers discuss how sgRNA-dCas9-EE can be utilized to regulate gene expression by targeting specific areas of the genes with a catalytically inactive Cas9 enzyme fused to epigenetic effectors, which allows for effective gene regulation without changing the actual sequence of the DNA, This paper further proves the importance of using a fused Cas9 to regulate gene expressions. However, this paper offers a new idea of fusing dCas9 and epigenetic effectors. Thus, instead of causing a double-strand break, epigenetic effector proteins are released to add or remove methyl groups to or from the DNA. This is crucial because it can allow for reversible changes. After all, the actual DNA sequence is not altered. It can also provide possibilities for epigenetic therapy. Moreover, since the actual Cas9 is inactivated, a possibility is that fewer irreversible errors will occur because it will only condense into heterochromatin or loosen the DNA into euchromatin.

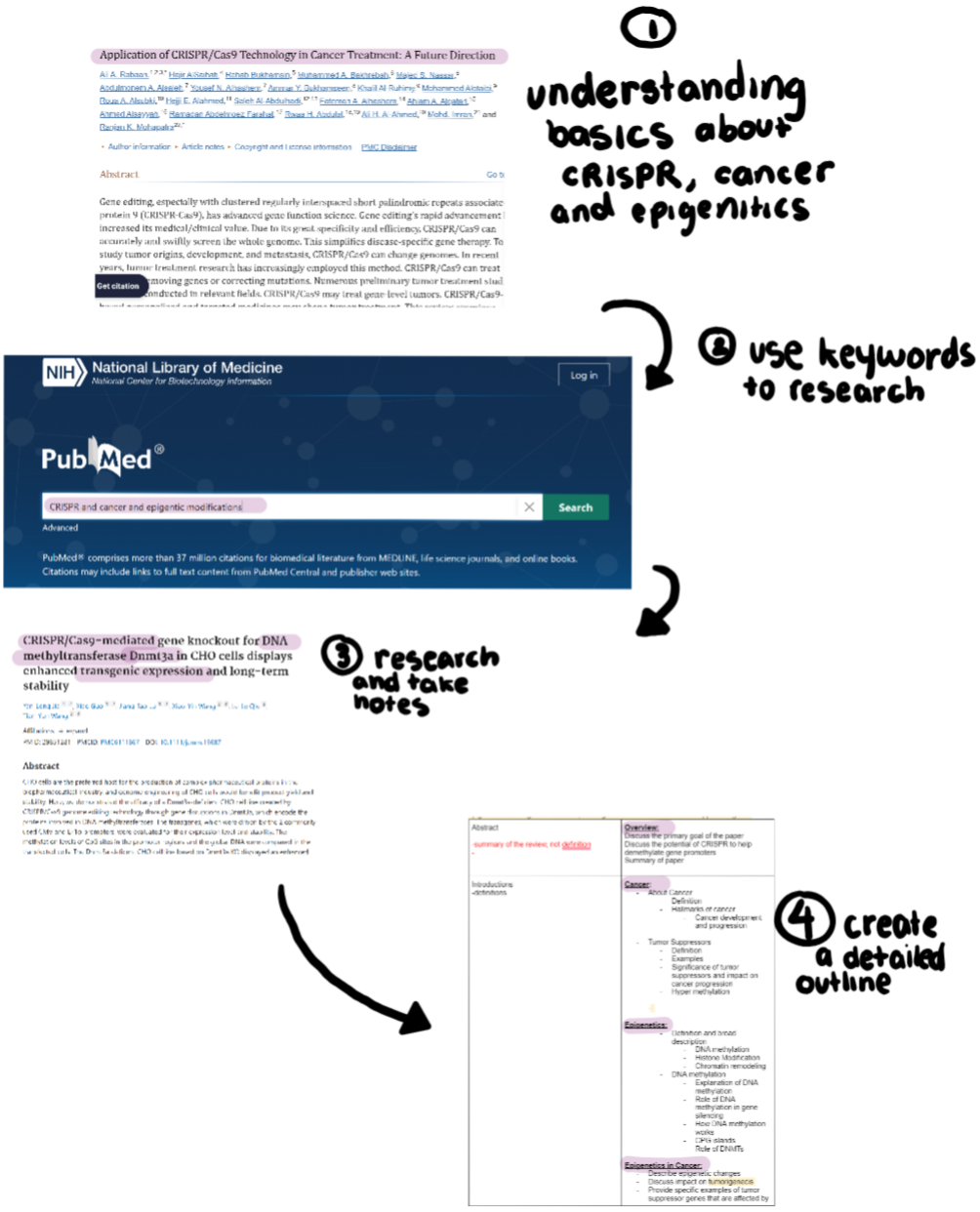

Fig. 5 Flowchart highlighting methods

This method is potentially significant to further consider by testing the sgRNA-dCas9-EE on multiple DNA by expressing and silencing certain genes to see the resulting traits while also ensuring there are no changes to DNA.

In 2021, Ocean Khajura investigated the capabilities of the CRISPR/dCas9 system for epigenetic editing (Ocean Khajuria, 2021)[5]. The paper's main goal was to identify the epigenetic alterations associated with different types of cancer and develop

ways to target and reverse modifications. The paper reviews multiple cases across different cancer types, such as liver, colon, prostate, and cervical cancer. The researchers found that for colon cancer, epigenetic editing with the dCas9 system successfully demethylated the SAR1, a tumor suppressor gene. This led to its reactivation because the DNA was loosened by the removal of the methyl group. They also found that for cervical cancer, the dCas9-TET1CD system effectively demethylated the BRCA1 gene, which also allowed for the reactivation of the tumor suppressor gene. They also found that for liver cancer, combining epigenetic effectors with the dCas9 system successfully suppressed the GRN gene, which has oncogenic factors. This paper demonstrates how the CAs9 system could be used to demethylate or methylate areas of interest in many different cancer types. This paper was necessary as it provided more trials of the use of Cas 9 and oncogenic factors to activate by demethylation, which is important for improving the accuracy.

In 2020, Imran Ansari discussed the uses of CRISPR/dCas9 systems for epigenetic editing in areas of cancer therapy in his paper (Imran Ansari, 2020)[6]. The goal of the paper is to identify the potential of using CRISPR/dCas9 as a therapeutic approach to cancer by investigating how the technology can be used to target the epigenetic marks associated with cancer. They used dCas9 systems fused with epigenetic effectors and specific epigenetic marks associated with cancer progression. They also researched the specific target areas for each type of cancer, including breast, liver, lung, colon, prostate, and cervical cancer. They used different epigenetic effectors such as DNMT3A, EZH2, and KRAB to modify methylation patterns, histone modifications, and other epigenetic marks. They also tested different in vivo delivery methods to introduce the system into cells. The researchers successfully used CRISPR/dCas9 to target epigenetic marks. They also analyzed and observed changes in gene expression and epigenetic marks that correlate with gene activation or inhibition. Finally, this paper highlights how dCas9-based epigenetic modifications could target oncogenes or tumor suppressor genes efficiently, which can help inhibit cancer progression. By providing more evidence on the successful use of Cas9 combined with epigenetic factors, the method is further supported, which allows for more accuracy, especially for the epigenetic effector DNMT3A, which was used multiple times accurately as observed in previous papers. However, this paper differs from some other examples because it provides an in vivo method that can improve accuracy. After all, one can visibly see the changes in the expression of different traits when experimenting rather than seeing changes in DNA structure in a lab.

Another paper written in 2021 by Muneaki Nakamura aimed to investigate the possible uses of CRISPR/Cas9 for precise epigenome modifications through methylation or demethylation (Muneaki Nakamura, 2021)[7]. Several CRISPR-based methylation and methylation mechanisms are explored in this paper. This paper used dCas9 systems as well. For demethylation, the researcher highlighted the use of dCas9-TET1 to reactivate tumor suppressor genes by removing DNA methylation marks. For methylation, the researchers emphasized the usage of dCas9-DNMT3A for targeted promoter methylation to suppress gene expression of oncogenes. They showed the successful application of dCas9 fusion proteins for targeted methylation and demethylation of specific gene promoters. This paper contributes crucial findings for future uses of CRISPR because it highlights specific genes like the DNMT3A and TET1 to improve the accuracy and possibility of using these protein combinations for demethylating cancer genes.

Additionally, in another paper, Anna Kazanets explored the role of epigenetic regulation in cancer and specifically focused on the silencing of tumor suppressor genes (Anna Kazanets, 2016)[8]. The paper also explores several different aspects of epigenetic regulation, like the role of long-coding RNAs (lncRNAs) in silencing tumor suppressor genes. The paper discovered that lncRNAs play a significant role in silencing tumor suppressor genes through histone modification like H3K27me3 or DNA binding. In conclusion, this paper highlights the potential of lncRNAs for regulating gene silencing. This paper is unique compared to other papers because it introduces the use of histone modification, which can also be through acetylation (the addition or removal of acetyl groups) of histones. This brings up an original idea, which wasn't brought up in previous papers, so it can be a beneficial idea to look into expanding possible ways of methylation with CRISPR and fusion proteins.

Another paper written by Jie Wang aimed to investigate the use of CRISPR/Cas9 for epigenetic editing by targeting de novo methylation of the MGMT gene promoter in 2022 (Jie Wang, 2022)[9]. The researchers pursued the development of a method to induce DNA methylation at specific CpG sites within the gene promoter. The paper's main goal was to understand the mechanism of epigenetic editing and its potential for tumor suppressor gene silencing. They used CRISPR/Cas9 to induce a homology-directed repair (HDR) to target specific sites in the gene promoter of MGMT. They then designed a double-strand break and repair template that had the desired methylation pattern. The long methylated dsDNA template functioned as a repair template for HDR. The researchers then analyzed the effects of the CRISPR/Cas9 system for mediated methylation on MGMT gene expression using both in vitro and in vivo methods. The key findings of this paper include the following. The paper discusses how the HDR method through CRISPR/Cas9 can maintain DNA methylation patterns in the MGMT promoter, which can be investigated for further research by experimenting with in vitro methods using the MGMT gene. They also found that using long methylated dsDNA templates can greatly improve the stability of DNA methylation rather than shorter templates. Additionally, the paper discussed how DNA repair and epigenetic factors generated heterogeneous methylation patterns, allowing DNA

methylation stability. In conclusion, this paper offers a unique approach to CRISPR/Cas9-mediated epigenome editing because the paper used the method of inducing a double-strand break, while other papers did not change the actual sequence of DNA. Therefore, this technique could be further explored. Additionally, this paper also looks into the effects of these methods on the promoter during transcription, which can directly affect expression without changing the structure of DNA. However, since the actual results were inconclusive, it is important to acknowledge the vast unknown area that researchers haven't covered yet with respect to CRISPR.This approach is different from other examples, and more experiments to test methylating promoter regions directly can be conducted in the future.

Another paper discussed the possibility of using a modular epigenetic toolkit, CRISPR/dCas9-SunTag, to target and epigenetically modify specific regions of the DNA. While trying to regulate certain genes using methylation techniques with with the Cas9 complex, the researchers faced several obstacles due to off-target effects, especially while working with dCas9-DNMT3A. This resulted in the failure of the use of DNMT3A as a fusion protein with Cas9. This is crucial to understanding the faults that still exist in CRISPR technology and the importance of ensuring an accurate representation of the accuracy of using fusion proteins.

In a recent paper in May of 2024, ScienceDaily explores emerging technologies and methodologies regarding using CRISPR for epigenetic modifications (ScienceDaily 2024)[10]. The paper highlights a Modular epigenome editing device, which allows for specific targeting of 9 chromatin marks at a region of the gene. This can allow researchers to know the impact of epigenetic modifications on specific chromatin rather than a general area, which can improve accuracy. Moreover, the paper also discusses a reporter platform that measures changes in gene expression, which can allow researchers to identify relationships between the chromatin marks and changes in epigenetic modifications. This is significant for overcoming main hurdles like inaccuracies with CRISPR and the inability to accurately track the effects of epigenetic modifications. Therefore, this new technology can open up potential for further exploration of methylation and demethylation of specific chromatin markers using CRISPR. Finally, the emerging trend to more accurate and specific methodologies for CRISPR-mediated epigenetic modifications can allow for future applications in cancer and other genetic diseases.

Discussion

Therefore, these papers show how CRISPR can be successfully applied to Cancer to demethylate or methylate parts of the CpG islands to restore the function of tumor suppressor genes and suppress the function of oncogenes. A majority of the papers decided on a technique that creates a Cas9 fusion protein called dCas9, which is Cas9 fused with proteins like DNMt3A and DNMT3B to edit and target known epigenetic marks that influenced oncogenes and tumor suppressors. They were then also able to silence these oncogenes and express the tumor suppressors again. This can prove to be very significant and useful in Cancer and CRISPR research because epigenetic editing in this way does not change the actual DNA sequence, which can allow for reversible effects. Many papers emphasized the significance of fusion proteins because they increase efficiency and accuracy, can allow for specific epigenetic modifications, and can target multiple genes. Therefore, this trend in technique can be useful for further research for identifying and modifying cancer-related genes. However, more trials and research should be done in this area because several unsuccessful cases show how this technique is not foolproof and that scientists should explore more fusion proteins like TET1 or MGMT.

Among the many benefits of the CRISPR/Cas9 system, it is important to consider some limitations of CRISPR technology, like off-target effects, delivery method challenges, Mosaicism, and ethical considerations. Off-target effects happen when CRISPR binds and edits a part of the DNA that is very similar to the expected target area, but it is not the actual DNA that needs to be changed. Moreover, specific to using CRISPR for epigenetic modifications, off-target effects can cause changes in the expression of certain genes because methylation and demethylation of DNA can completely stop or start gene expression. Thus, the demethylation of a gene that codes for a harmful protein can cause it to be over-expressed, while the methylation of a gene that codes for a necessary defense protein can cause it to be under-expressed. Especially with cancer, this can cause tumor suppressors to be reduced and mutated oncogenes to be expressed. Therefore, this can be detrimental because it can cause unwanted changes and worsen the function of cells in cancer. Thus, the papers in this study attempted to mitigate off-target effects by increasing the specificity of the target using modified Cas9 systems like d cas9 and using multiple guiding RNAs to increase specific binding. Additionally, the delivery method challenges the restraint of the function of CRISPR because effectively delivering CRISPR into the cell can be difficult. Additionally, large insertions are incredibly challenging because of the limited capacity of CRISPR delivery methods. Mocaism, which is when the change in DNA only causes the change in the first cell division and not further, can be important if one is trying to avoid a mutation or part of the DNA. Finally, ethical considerations should be examined as well because, generally, being able to suppress oncogenes and recover tumor suppressor genes can bring up ideas of suppressing unwanted genes and expressing beneficial genes through hypo-methylation and hype-methylation, which can bring up ethical concerns. Therefore, it is of utmost importance to understand the possible limitations while looking at the methods of successful hypo-methylation and hyper-methylation of oncogenes and

tumor suppressor genes. Furthermore, it is also important to consider ethical implications like informed consent, boundaries, and accuracy. Informed consent is important because, ultimately, the patient should be in complete control to decide whether they would like a treatment, and they should be in a position to fully understand it. Additionally, ethical boundaries are important to avoid monetary greed and ideas like CRISPR babies, which is the idea of editing genes to instill desired traits in one's offspring. Finally, it is crucial to ensure that the CRISPR technology is as accurate as it can be without causing more harm than it helps by monitoring changes in trends of accuracy and efficiency. Therefore, while CRISPR can be really beneficial for the epigenetic modification of cancer genes, potentially adding to the journey toward a cure for cancer, it is important to be mindful of the limitations that researchers could face while investigating the ideas discussed in this paper further since the CRISPR technique is not foolproof. Currently, researchers are working with new technology that identifies specific epigenetic markers in the chromatin. This provides a potential for finding new marks to target and question past conventions, which can increase accuracy and prevent the possibility of off-target effects. Another emerging technology that has been proven to overcome hurdles like off-target effects when dealing with CRISPR and epigenetic modifications is nanopore technology, which can monitor the epigenetic modifications live. This can be further explored in the future or improving CRISPR and expanding possible uses while also acknowledging ethical considerations.

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