

Exploration of the application of gene editing technology in the treatment of colorectal cancer, taking CRISPR technology as an example for editing FAT4 genes

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Abstract. Colorectal cancer, the fourth most common cancer in the world, is the most common digestive tract tumor. At present, conservative treatment is still the main treatment method for colorectal cancer in clinical practice. Among the pathogenic factors of colorectal cancer, genetic factors need to be emphasized. Currently, many studies have focused on pathological studies of related gene mutations, mostly focusing on genes such as p53 and APC, and their molecular biological reactions have been thoroughly studied. However, research on other pathogenic genes is still lacking, and there is limited research on specific treatment methods. With the birth of CRISPR-Cas9 technology, applying of gene editing technology in the field of cancer has made breakthrough progress. This article will consider combining gene editing technology with related gene mutations in colorectal cancer, attempting to explore the possibility of applying gene editing technology in the field of cancer treatment, proposing feasibility for new treatment plans for colorectal cancer, and also providing new treatment ideas for future cancer treatment research.

Keywords: The paper must have at least three keywords: Colorectal cancer, gene editing, mutation, FAT4, CRISPR-Cas9.

1. Introduction

Colorectal cancer is a common digestive tract malignant tumor. Its incidence rate ranks fourth in all tumors, only second to gastric cancer, esophageal cancer and breast cancer. There are 8159 cases in the United States, mainly among middle-aged and elderly men. Among all the pathogenic factors of CRC, genetic factors account for a large proportion, with 21060 related genes and 337904 mutation sites [1]. The representative gene mutation site studied in this article is FAT4, which ranks 7th in the number of mutations in all cases.

This article aims to analyze the possibility of using gene editing technology to perform gene editing at mutation sites in CRC, reducing the proportion of genetic factors in the cause of CRC, and thus providing new methods for reducing the incidence of CRC. At present, the clinical treatment methods for CRC are mostly surgical surgery and conservative treatment (medication), which cannot reduce the likelihood of recurrence [2]. However, gene editing can modify the relevant pathogenic mutation points to normal cells, reducing their pathogenicity, and achieving the effect of complete eradication of CRC. Gene editing technology still has a certain degree of uncertainty, making it difficult to achieve

100% accuracy in gene editing. There is a possibility of editing errors, which may actually lead to normal genes becoming pathogenic genes. At the same time, gene editing technology faces certain ethical and social issues, which are also the difficulties that gene editing technology is currently facing in clinical practice [3-5].

In previous articles, many scientists have explored gene editing technology's advantages and disadvantages and the possibility of its use in the treatment of diseases, but almost no one has applied it to practical clinical cancer treatment plans [3-5]. Most of them have applied gene editing technology to the construction of cell membrane types in basic medical research, and cancer is precisely caused by changes in cell gene mutations at the cellular level. Therefore, it is necessary for us to explore the possibility of gene editing technology in cancer. This article focuses on CRC, taking specific mutation sites and gene editing technology as examples to show the feasibility of gene editing technology in treating CRC and propose the feasibility of new treatment plans for CRC.

This article will analyze the specific mechanisms of CRC, especially the pathological mechanisms caused by gene mutations, and introduce several currently most representative gene editing techniques. Finally, the editing possibility of CRISPR technology in CRC-related mutation site FAT4 will be used to intuitively demonstrate the possibility of gene editing technology in the treatment of CRC.

2. The Pathogenic Mechanism of CRC

CRC, the most common malignant tumor in the gastrointestinal tract and the fourth most common cancer in the world. As the cancer increases, symptoms such as changes in bowel habits, bloody stools, alternating diarrhea and constipation, and local abdominal pain may appear. In the later period, there may appear systemic symptoms such as weight loss and anemia [1]. This disease mostly occurs in men over middle age, with the most common occurrence occurring between the ages of 40 and 70. Through World Cancer Epidemiology Survey, the incidence rate of colon cancer is high in North America, New Zealand, Australia, Western Europe and other places, but low in Finland, Japan, Chile and other places. It believes that the geographical distribution is related to the residents' eating habits, and the incidence rate of high fat diet would be higher [6]. Among all possible sites of intestinal disease, the most common site are the sigmoid colon and rectum, while the rest are sequentially seen in the ascending colon, cecum, transverse colon, and descending colon. The majority of cancer tumors are adenocarcinoma, with some being squamous cell carcinoma and mucinous carcinoma. This disease can be spread to other tissues and organs by blood circulation, lymphatic, and direct spread. Diagnosis can be made based on clinical manifestations like X-ray barium enema or fiberoptic colonoscopy [1].

In plenty of clinical cases, fact has been found that colon polyps may undergo malignant transformation, with papillary adenomas being the most prone to malignant transformation, reaching up to 40%. While the patients with familial polyposis, the incidence of cancer transformation is seen higher, indicating a close relationship between colon cancer and colon polyps. In addition, some chronic ulcerative colitis can be sophisticated with colon cancer, and the incidence may be 5-10 times higher than that of the normal population. The reason for the colon cancer's occurrence can be in connection with chronic inflammatory stimulation of the colon mucosa. It is generally believed that cancer may occurs through the inflammatory polyp period during the inflammatory proliferation process [6].

In the later period of tumors: persistent small amounts of bloody stools can cause anemia; Chronic progressive malnutrition, anemia, and local ulceration, as well as toxic symptoms result from the absorption of infectious toxins, giving rise to cachexia, general weakness, mental exhaustion and emaciation in patients. Acute perforation can cause acute peritonitis. Liver enlargement, neck, ascites and supraclavicular lymph node enlargement often indicate advanced stage of the tumor and metastasis [1].

2.1. The mechanism of CRC-related gene mutations

Among all cases of CRC, 20-30% of CRC patients have a family history of CRC, so genetic factors have become a major factor that cannot be ignored in the prevalence of CRC [1]. In recent years,

research has identified the characteristics of the main molecular changes that occur in CRC, indicating that each patient's tumor exhibits two to eight gene mutations. A large amount of experimental evidence shows that CRC can originate from intestinal stem cells' malignant transformation or the malignant transformation of intestinal cells which acquiring stem cell characteristics after malignant transformation, leading to normal colonic epithelium's pathological transformation into adenomatous polyps and developing into invasive cancer ultimately. Tumor suppressor genes and oncogenes' mutations are believed to give cell proliferation advantages and contribute to the development of malignant phenotypes. Meanwhile, mutations in intestinal stem cell genes may be related to chemotherapy resistance, radiation resistance, and recurrence [6, 7]. For example, the inactivation mutation of two copies (alleles) of the adenomatous polyposis of the large intestine (APC) gene (tumor suppressor gene on chromosome 5q) marks the occurrence of CRC. The mutation of APC may lead to hereditary familial adenomatous polyposis syndrome, which can lead to a large number of polyps in the intestine and, if not treated promptly, can lead to CRC. In addition, mutations with dysregulation of the K-ras oncogene are also considered one of the possible causes of colon cancer formation. The loss of heterozygosity in chromosome 18's long arm (18q) of occurs in the later stages of development from adenoma to cancer and is generally considered to manifest as poor prognosis. The mutation of the tumor suppressor gene p53 on chromosome 17p is considered a late stage phenomenon in the development of CRC. This mutation may enable growing tumors with multiple genetic changes to evade cell cycle arrest and apoptosis, thereby affecting the possibility of CRC occurrence. Recently, new studies have shown that DNA repair genes may also affect the occurrence of CRC. The research results indicate that due to germline mutations in the hMSH2, hMLH1, hPMS1, or hPMS2 genes, DNA mismatch repair may lead to hereditary non polypic cancer, and patients with such diseases exhibit microsatellite instability, which is replication error positivity.

2.2. The pathological mechanism of CRC associated with gene mutations

In the past few years, the understanding of CRC has made significant progresses, which allow us to study the relevant molecular and cellular mechanisms based on tumor progression. Colon cancer progresses from normal colonic epithelial cells to adenomatous polyps, and then to invasive cancer. This progression from adenoma to cancer enables researchers to construct ordered molecular models, including genetic changes and cell clone proliferation. In these models, there are three different types of mutated genes: tumor suppressor genes, oncogenes, and DNA repair genes. Below, there are some specific examples to be discussed:

In the study of hereditary colon cancer syndrome, it was found that there is APC, a tumor suppressor gene, in familial adenomatous polyposis, and mutations in this gene were observed in these sporadic cancers. In sporadic cases, loss or inactivation of the APC gene was observed in somatic cells. More than 90% of APC gene mutations inactivate the gene, leading to premature termination of transcripts. At the same time, other studies have also demonstrated that mutations in the APC gene or deletions of remaining alleles have been observed in somatic cells associated with familial adenomatous polyposis, such as duodenal adenomas and gastric peripheral cancer. APC protein may also regulate the cell cycle by regulating the activity of the cyclin cyclin-dependent kinase (CDK) complex. Therefore, mutations in APC will significantly increase the likelihood of cancer transformation in cells, making patients more likely to develop CRC [8].

Through molecular genetics research, the short arm of chromosome 17 (17p) was found as one of the most common allele loss sites in CRC, and it was observed that the smallest deletion region on 17p contains the p53 gene. In cases where a 17p gene mutation occurred, sequence analysis of p53 revealed that the allele of the p53 gene often mutated, indicating that p53, a tumor suppressor gene, present in this region. The partial biochemical function of the protein product of the p53 gene has been studied, and the p53 protein has the ability to bind specific DNA sequences and activate downstream gene transcription. Its representative downstream binding target p21 has the ability to inhibit cyclin B-CDC2 and other cyclin CDK complexes and is a key protein in regulating cell metaphase. Mutations in the P53 gene may lead to overexpression of p53 cells, reverse activation of p21 protein, leading to

cell cycle arrest, allowing tumor cells to escape normal growth arrest and apoptosis, leading to the occurrence of cancer [9].

3. Gene editing technology

Gene editing refers to the process of modifying specific targets of an organism's genome by gene editing techniques. Efficient and precise implementation of gene deletion, insertion, or replacement, thereby altering its phenotype characteristics and genetic information. It is a revolutionary biotechnology used to modify the genome of organisms. Gene editing technology can achieve targeted "editing" of specific target genes to change the sequence, expression level, or function of target genes or regulatory elements, including the addition, deletion, and replacement of specific DNA fragments [4]. Through gene editing technology, humans can study the function and mechanism of genes, treat genetic diseases and cancer, improve crops and animals, and even create new forms of life [3]. The basic principle is to induce double-strand breaks (DSBs) at specific targets, thereby initiating two main DNA repair mechanisms within cells: homologous recombination (HDR) and non-homologous end junction (NHEJ). HDR relies on templates for targeted repair, which can achieve precise insertion, deletion, or base replacement of specific localization points, making it more accurate. NHEJ does not rely on templates to directly connect the two ends of DNA, and can effectively generate insertion or deletion of fragments of different lengths at the DSB site, usually leading to gene dysfunction, fast but imprecise [4, 5]. At the end of the 20th century, various gene editing techniques based on endonuclease mechanisms were developed to achieve precise localization of specific gene loci [16]. Below is a brief introduction to several gene editing techniques:

The Cre lox system is a technology that utilizes the Cre recombinase of bacteriophage P1 and the loxP recognition site for site-specific gene recombination. It can achieve gene knockout, insertion, flipping, and translocation. This system has the following advantages: simplicity, high efficiency, strong specificity, wide application, and controllable spatiotemporal specificity, but it also has limitations: off-target effect, Cre toxicity, incomplete shear, etc [10, 11].

Zinc finger nuclease technology (ZFN) is a gene editing technique that utilizes artificially designed fusion proteins [12]. Zinc finger proteins are a class of transcription factors widely present in eukaryotes, capable of recognizing and binding to specific DNA sequences within the zinc finger protein domain [13]. Endonucleases can cleave the DNA double-stranded endonuclease domain, thereby causing DNA double-stranded breaks at specific locations. ZFNs have strong sequence specificity, high cleavage efficiency, and a wide application range, but the design and construction of ZFNs are complex and expensive and may cause non-specific cleavage or cytotoxicity [12, 13].

Transcription activating factor effector (TALE) is a type of transcription factor derived from the genus of plant pathogenic bacteria, which can regulate the expression of plant genes by interacting with DNA sequences in specific gene promoter regions in plant cells [12]. Transcription activator-like effector nucleases (TALENs) are artificial proteins formed by fusing the TALE DNA binding domain with the Fok I endonuclease domain. When two TALENs tie to DNA separately, they form a dimer between them and cleave the DNA double-strand through the Fok I endonuclease domain, forming a double-strand break [15]. The design of TALENs is simple, with a wide range of recognition sites, high shear efficiency, and low toxicity. However, TALEN proteins are large and difficult to transfect into cells and may cause non-specific cleavage or cytotoxicity [12, 13].

3.1. CRISPR-Cas9

This is the most commonly used gene editing technique in research currently [12, 13]. It utilizes a natural immune system from bacteria: certain bacteria, upon being attacked by viruses, are able to reserve a small segment of virus genes in DNA in a storage space called CRISPR [16]. If encountering virus invasion again, bacteria can identify the virus through the reserved and written fragments, cutting off the virus's DNA and rendering it ineffective. The basic principle of this technology can identify target genes by artificially designed single-stranded guide RNA (sgRNA) and guide Cas9 protease to effectively cleave DNA double strands, which will form double-strand breaks. After being damaged,

DNA's repair function will cause gene knockout or knocking in. CRISPR, a repeating sequence within the genome of prokaryotes, is mainly composed of a leading sequence, repeating sequence, and spacer sequence. The Cas gene locates near the CRISPR gene or disperses in other parts of the genome, and the protein which is encoded by this gene will come into contact with the CRISPR sequence region. Thus, this gene is named CRISPR associated gene [17]. CRISPR-Cas9 technology has the advantages of being faster, cheaper, and more precise compared to previous gene editing technologies, and also has broader potential applications. Its disadvantage is that non-specific cleavage or off-target effects may occur, and the Cas9 protein is relatively large, which makes it can't transfect certain cells well [3].

3.2. Applying of CRISPR-Cas9 in CRC (using FAT4 as an example)

This article will take FAT4 as an example to explore the potential application of CRISPR-Cas9 in CRC. Human FAT family (FAT-4) protein is a homologous protein of *Drosophila* adipose tissue, which is involved in tumor suppression, planar cell polarity, and cell growth. In the last few years, tumor-related studies have shown that FAT4's downregulation can be connected to the pathogenesis of various malignant tumors, and it has also been recognized as a tumor suppressor factor for various cancers, which helps to inhibit cell proliferation and invasiveness, thereby inhibiting tumor cell metastasis. In particular, FAT4 is newly went down as a biomarker for the prognosis of breast cancer [18]. The role of FAT4 in the pathogenesis of cancer may be connected to Hippo signaling pathway, human papillomavirus infection, cell junction assembly, PI3K-Akt signaling pathway, protein tyrosine kinase activity, etc. Through analysis of the data in the TCGA database, we found that among all patients with CRC and genetic mutations, FAT4 accounted for the seventh most missense mutation among all mutated genes, and the overall survival cycle of patients with FAT4 mutations significantly decreased. The mutation of FAT4 may reduce the survival rate of patients. Therefore, we consider modifying the mutated FAT4 gene through gene editing technology to improve patient survival cycle and prognosis.

The first step is to search for suitable mutation sites. Select exploration in the GDC data portal of the NIH website and enter FAT4 in the Gene database. In the detailed information of the FAT4 gene, 215 mutation sites can be seen. When selecting mutation sites, the most important consideration needs considering is the changes in protein structure and function caused by their mutations, as well as the potential cancer risk associated with them. Therefore, after comprehensive consideration, the site chr4:g.125317027G>C was chosen. This site is a missense mutation that presents high risk in both SIFT and PolyPhen indicators. The changes in protein structure and function caused by its mutation are highly likely to affect the anti-cancer function of the protein, leading to the occurrence of cancer. Due to its mutation, glycine at the corresponding site on the protein changes to arginine.

Next, proceed with the design of sgRNA. Firstly, import the FAT4 gene onto the workbench on the benching website. After importing, the specific sequence of FAT4 will exist on the work interface. By clicking on the sequence map, the mutation site 125317027 can be found in the 125317000-125317050 segment. Next, click on the CRISPR button on the toolbar on the right to enter the specific steps of constructing sgRNA. After obtaining sgRNA and determining its PAM region, Cas protein will be used to remove the mutation site at the site, which can successfully form DNA breaks on the double strand of FAT4. In the subsequent cell repair process, frameshift mutation is used to remove the mutated gene and repair the natural gene, repairing the mutated FAT4 gene to a normal FAT4 gene [5, 20].

4. Conclusion

Firstly, this article demonstrates through the use of CRISPR technology for site-specific mutations in the FAT4 gene that gene editing technology can modify mutation sites on related mutation genes in CRC, thereby modifying the normal physiological function of cells, in order to reduce cancer incidence, improve the prognosis of cancer treatment, and improve the overall survival cycle of patients. This proves the feasibility of gene editing technology in the treatment of CRC, propose

feasibility for new treatment plans for CRC and provide new treatment ideas for future cancer treatment research. In future cancer treatment, similar therapies can be considered, including gene editing as a treatment method. Of course, there is a lack of relevant clinical data to support the expectations of this article, and the ethical and social issues brought about by gene editing technology cannot be ignored

References

- [1] <https://portal.gdc.cancer.gov/>
- [2] Ugo Testa,* Elvira Pelosi, and Germana Castelli 2018 CRC: Genetic Abnormalities, Tumor Progression, Tumor Heterogeneity, Clonal Evolution and Tumor-Initiating Cells
- [3] Meryem Alagoz and Nasim Kherad 2020 Advance genome editing technologies in the treatment of human diseases: CRISPR therapy (Review)
- [4] Weilin Zhou, Jinrong Yang, Yalan Zhang 2022 Current landscape of gene - editing technology in biomedicine: Applications, advantages, challenges, and perspectives
- [5] Margaret Waltz, Eric T. Juengst, Teresa Edwards 2021 The View from the Benches: Scientists' Perspectives on the Uses and Governance of Human Gene-Editing Research
- [6] Ceres Fernandez-Rozadilla, Maria Timofeeva, Zhishan Chen,et al 2023 Deciphering CRC genetics through multi-omic analysis of 100,204 cases and 154,587 controls of European and East Asian ancestries
- [7] Peng Pan, Jingnan Li, Bo Wang 2023 Molecular characterization of colorectal adenoma and CRC via integrated genomic transcriptomic analysis
- [8] Lu Zhang, Jerry W Shay 2017 Multiple Roles of APC and its Therapeutic Implications in Colorectal Cancer
- [9] Magdalena C Liebl, Thomas G Hofmann 2021 The Role of p53 Signaling in CRC
- [10] Allisa J Song, Richard D Palmiter 2018 Detecting and Avoiding Problems When Using the Cre-lox System
- [11] Michael Bordonaro 2009 Modular Cre/lox system and genetic therapeutics for CRC
- [12] Thomas Gaj, Charles A Gersbach, Carlos F Barbas 2013 ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering
- [13] Edyta Janik, Marcin Niemcewicz, Michal Ceremuga 2020 Various Aspects of a Gene Editing System-CRISPR-Cas9
- [14] Dana Carroll 2011 Genome engineering with zinc-finger nucleases
- [15] Anuradha Bhardwaj, Vikrant Nain 2021 TALENs-an indispensable tool in the era of CRISPR: a mini review
- [16] Dana Carroll 2017 Genome Editing: Past, Present, and Future
- [17] Alexandre Loureiro, Gabriela Jorge da Silva 2019 CRISPR-Cas: Converting A Bacterial Defence Mechanism into A State-of-the-Art Genetic Manipulation Tool
- [18] Weili Mao, Jiajing Zhou, Jie Hu, et al 2022 A pan-cancer analysis of FAT atypical cadherin 4 (FAT4) in human tumors
- [19] www.benchling.com