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The Application of CRISPR/Cas9 in Cancer Immunotherapy

Jiale Yu

Columbia International College, Hamilton, Canada
2024011022xzk@cic.care

Abstract:

Currently, Cancer is the number one killer of human health. The death rate from cancer is increasing year over year as the population ages and grows, particularly since 2019, when there were almost 10 million cases globally recorded by the World Health Organization every year. In light of this trend, it is imperative to continue developing medications and to update and iterate on anti-cancer therapeutic technologies. CAR-T cell therapy, microphage-based therapy, oncolytic virotherapy, and checkpoint blockade therapy are the most popular cancer treatments, but a few patients still cannot tolerate these therapies. A post-optimized strategy is to use gene editing to alter the characteristics of cancer treatment. New therapeutic options for the treatment of complex disorders have become available with the development of CRISPR/Cas9 gene editing technology in recent years. This method is widely used in market research for gene knockdown, endogenous gene expression, and chromosome locus markers because of its accuracy and efficiency in processing harmful gene fragments. Also, it is extremely valuable from a medical standpoint when researching immunomodulator resistance. Despite its great potential, there remain concerns over the technology's usefulness and efficacy and negative prognostic reactions. This review primarily focuses on the current use of CRISPR/Cas9 in cancer immunotherapies.

Keywords: CRISPR/Cas9; cancer immunotherapy; oncolytic virotherapy; CAR-T therapy.

1. Introduction

With one case of cancer killing a person worldwide, on average six people, cancer ranks as the third-greatest cause of mortality all over the world. It is a disease with a significant growth rate, and the growth process is accompanied by the failure of cells's inhibitory factors and the accompanying inflammation, tumor escape in the immune system, and other occurrences. When the epigenome's inheritance is affected, oncogenes are created, adapting to the host's environment through further genetic mutations. Cancer is made up of a high number of abnormal cells that spread uncontrollably and infect healthy areas, causing devastation. When people have cancer, there are many side effects that come with it, such as inexplicable discomfort and unexplained weight loss. More specifically, those odd bumps on the body might indicate maglicant cells rather than be the result of a bacterial infection. According to continuous data from ongoing tracking by the International Agency for Research on Cancer, 23.8 individuals will suffer from diverse cancer diseases prospectively, and by 2030, there will be roughly 13 million people killed for it [1].

Since the 1970s, advances in genetic engineering technology have raised the possibility of successfully treating cancer. The efficacy of cancer immunotherapy is being studied more and more as knowledge about the human immune system, which is composed of lymphatic and white blood cells, grows. Cancer cells are stopped from proliferating by using the immune system's built-in bacterial capabilities. These days, the most popular ones include the vaccine, adoptive cell treatments, CAR technology, and checkpoint inhibitors. Their appearance provides a fresh interpretation of life's creativity and malleability. But while their effectiveness and safety are a constant source of worry, CRISPR gene editing technologies simplify the design process to avoid these issues with economic and scientific utility. Global scientists now possess an abundance of first-hand knowledge about the human genome, mostly due to the advancements in high-throughput sequencing technologies. In order to edit the cas9 protein and gradually cut particular DNA double strands for usage in mammals, signal-guided RNA based on binding of crRNA and tracrRNA was used between 2012 and 2013 [2]. The functional mechanism of CRISPR-Cas9 cutting DNA in vitro was demonstrated during the same era as Charpentier and Doudna worked to extract cas9 from streptococcus thermophilus and streptococcus pyogenes. This discovery is a landmark of significance. Prior research catagorized Cas9 as the sole necessary component

in type II, which is one of the three CRISPR/Cas system classifications already defined. TracRNA, crRNA, and Cas9 come from the CRISPR-Cas9 system. A crucial part of breaking some DNA double strands is played by the multistructural protein cas9, which is composed of HNH nucleases and RuvC nucleases [3]. tracrRNA and crRNA are intimately associated because the prepart of them can connect each other to form signal-guided RNA (sgRNA), causing it to mature swiftly with the aim of exerting its accompanying sequence to find exogenous DNA and direct CRISPR-Cas9 gene editing [4]. Nevertheless, sgRNA and cas9 cannot completely combine to cut the target sequence in the absence of an activation signal from PAM. It was discovered that the targeted DNA containing PAM would create a complex structure with sgRNA in the sp-Cas9 structural analysis experiment [5]. DNA's self-repair mechanism is triggered in two different ways following the breakage of the double strand, which are NHEJ and HDR. When a gene is lost or deleted, leading to genetic mutation, homologous DNA repair is more accurate than nonhomologous end joining.

By combining the CRISPR/Cas9-based Sherlock cancer diagnosis system and utilizing it to extract numerous genetic alternations to create a virtual tumor model, treatment accuracy and healthcare worker efficiency are significantly increased. Furthermore, it has been extensively employed in the treatment of lung cancer, cervical cancer, colon cancer, and other significant illnesses. Cancer cells are also caused by certain carcinogenic viruses, and Crispr/Cas9 technology makes it possible to prevent or even eradicate the virus's source, which includes HPV and HBV. Despite the fact that CRISPR/Cas9 would be affected by off-target effects, there may be significant biological advantages to using it to treat infection-related cancers and other illnesses. CRISPR/Cas9 is presented in this review, along with examples from applicable sectors such as checkpoint blockade therapy and CAR-T cell therapy, oncolytic virotherapy, and micro-based therapy, as well as discussing developed prospects.

2. The Application of CRISPR-Cas9 in Oncylotic Virotherapy

In addition to serving as the foundation for the distribution of viral vectors, oncotic cytolytic viruses have the ability to selectively destroy tumor cells while sparing healthy ones. The second generation of oncolytic herpes virus, talimogene laherparepvec, achieved an immune response rate of nearly 30% [6]. Adenovirus is a kind of DNA double-stranded virus in oncolytic virus. Its noteworthy characteristic is that it will remain infected even if the cell divides, and it will resist being randomly inserted into the host cell to prevent insertion-related changes [7]. The researchers inserted Crispr-cas9 into adult organisms via adenoviruses in order to rearrange their chromosomes. The simulation generates a human cancer model in an organism using the GMEE Tumor Assisted Analysis tool, which has significantly slowed down the work of numerous gene editors. An example of this was in a NASA animal model, where adenoviruses were able to continue editing Pren despite the liver's cellular immunotoxicity, causing hepatic puffiness in just four months.

Moreover, oncolytic virus tumor selectivity is enhanced by CRISPR-Cas9. According to a report, activation of the E1B gene attenuated adenovirus cells to improve the cell's anti-tumor immune response, and the gene itself, which codes for the 55 kilodalton (kDa) protein, inactivated the p53 gene to avoid epigenetic alterations [8, 9].

Otherwise, crispr-cas9 is utilized to alter HSV in order to treat colon cancer. According to the experimental data, gRNA-guided cas9 transfer transfection leads to DNA repair with an efficacy that is approximately 5.6 million times higher than DNA repair achieved by means of viral plaques. O-HSV1 was created by deleting the ICP345 and ICP347 genes using crispr-cas9, with HSV-1 being chosen as the parent virus. O-HVS1 has a higher replication capability than wild-type HSV1. The mice in this group then lived for 39 days after taking an additional step and developing a gene to control the expression of IL12 and CXCL11 as well. as the virus. The mice in the control group survived only 24 to 30 days. INF-y, a derivate after the combination of IL12 and CXCL11, increases the toxicity of cells and prolongs mice's lives, illustrating great potential in treating colon cancer.

The cancer virus has undergone several modifications, and its capacity to infect cell populations has been used to infect tumor cells, becoming an excellent viral vector for CRISPR/Cas9 delivery. CRISPR/cas9 cannot target the NARS gene exactly in the traditional virus vector but rather has a better effect on myxoma virus (MYXV) in xenotransplantation of mouse embryonic rhabdomyosarcoma [4]. NASA genes are more likely to be inactivated, and tumor growth or proliferation is less likely following CRISPR-Cas9 mediation. The OV virus can find numerous virus sequences at once and has a high capacity of loading, offering those sickles more hope and choice. Compared to conventional methods, the more labor-intensive CRISPR-Cas9 system presents a significant opportunity for the development of therapeutics using oncolytic viruses.

3. The Application of CRISPR-Cas9 in Checkpoint Blockade Therapy

Drug-resistant conventional cancer cells arise, while the immune system is crucial for monitoring cancer, certain

tumor cells may nevertheless manage to escape their control. Immune check point therapy has made significant strides in the previous 10 years and caught the interest of pharmaceutical corporations and the relevant medical community. In particular, there have been substantial clinical breakthroughs in checkpoint drugs for PD-L1, PD-1, and CLTA-4 [10]. CRISPR/Cas9 plays a vital role in knockdown the PD-L1 or CTLA-4 in cytotoxic T lymphocytes, which is mainly responsible for killing tumor cells (CTL). The signal delivered by PD-L1 or CTLA4 would influence the normal function of CTL. Therefore, suppressing the expression of PD-L1 and CTLA-4 can enhance the immune cell responses of the sick. As a report showed, mice in studies where CTLA-4 and PD-1 genomes were deleted had longer lifespans than the group that did not get treatment. In addition, the group that had these two genes deleted was able to emit more TNF-a and INf-y, two killer cytokines, than the experimental control group. The lymphatic system secretes INF-y, which has potent anti-tumor immunological properties. It is evident that knocking down PD-1 and CTLA-4 is an alternative for research associated with drug discovery and development.

PD-L1 gene knockout is another topical field. The gene segment of PD-L1 was accessed to live longer than the control group. Meanwhile, the destruction of PD-L1 can produce the proliferation of tumor-infiltrating cells, promoting which chemokine molecules are regulated to control the metastasis and expansion of tumor cells. Besides, a medical project proves that using the CRISPR/Cas9 system to kick out PD-L1 in CTL can generate more immune responses to play against the invasion of tumor cells, which forms a highly effective way to treat Epstein-Barr virus-positive gastric cancer [11]. Overall, CRISPR/Cas9 is used to recognize target checkpoints and modulate the desired effector on them, providing guidance on improving intervention strategy on cancer immunotherapy progress.

4. The Application of CRISPR-Cas9 in CAR-T Cell Therapy

CAR-T cell therapy has been applied in many fields of treatment, especially for blood vessel solid tumors. The CAR protein is a fusion protein with multiple domains, but it is mainly acted upon by scFV. This chain structure assists T cells with recognizing tumor antigen, avoiding the suppression of T cell activation. In the recent clinical trials, CAR-T cell therapy demonstrates favorable therapeutic effects on therapying acute myeloid leukemia, and the remission rate is astonishingly close to 100 percent [12]. However, induced CAR-T cells can activate some checkpoint expression, such as PD-1, which can limit the partial antitumor ability of the human immune system. In the meantime, it may cause the CRS, reducing the cytotoxicity of antitumor cells. Additionally, the TCR alpha and beta chains may sometimes recognize duplications that cause GVHD, leading to rejection of leukocyte antigens [13]. Therefore, it is necessary and imperious to design ready-made CAR-T cells to overcome risks from the tumor microenvironment.

CRISPR/Cas9 was used to modify the gene expression in primary T cells. By knocking out the death proteins, which include PD-L1 and PDCD1, in T cells, scientists can reduce the immunogenicity of T cells and greatly decline cytotoxicity, which means T cells have more opportunity to be transplanted into the human body. In 2019, in mouse glioma models, CRISPR-Cas9-mediated anti-CD133 t cells can greatly improve cytotoxicity and cell proliferation [14,15]. The production of general-purpose CAR T cells in the recent study was formed by CRISPR-Cas9 knockout of t cell receptor-alpha and B2M. Their main function is to weaken the host's immune recognition ability to isolate and extract relevant cells. In mice with the gene defect, the anti-CD19 CAR T cell after the deletion of the two genes retained the function of the immune response in the original mouse and did not produce GVHD [14]. What's more, the Fas receptor has been shown in clinical experiments to be involved in T cell effects, and its combination with its ligand can induce apoptosis of other CAR T cells. However, CAR-T cells were heavily edited with crispr-cas9 to lose almost all Fas receptors, which greatly improved the efficiency of anti-tumor cells. Likewise, CRISPR/Cas9 is used for mass screening of drug molecules, providing a new way to reduce the risk of CRS happening. The continuous improvement of methods targeting the car series and its ability to accurately destroy genes has raised the possibility of cancer being cured.

5. The Application of CRISPR-Cas9 in Microphage-Based Therapy

The escape of tumor cells in the immune system is called tumor immune escape, and the purpose is to prevent digestion by immune cells such as macrophages, which have a strong ability to attack and phagocytosis. CD47 is a typical escape object. It is still able to formulate an escape strategy when the macrophage signals to engulf. It tries to connect with alpha, a signal-regulating protein on macrophages, and then bypasses the phagocytosis mechanism directly. Phosphorylated products such as SHP-1 and 2 can inhibit the accumulation of phagocytic muscle proteins at the contact between phagocytes and tumor cells, thereby avoiding their own fate of absorption [16].

Before CRISPR/cas9, scientists had developed ways to block CD47 and macrophage regulatory proteins. At pres-

ent, the more commonly used method is to use inhibitors against cd47 protein expression. Although the results after use are relatively consistent with treatment expectations, overuse can activate and prolificate potentially threatening toxicity that affects the cell death cycle [17]. CRISPR-cas9 knocks out SIRP-a, reduces the inhibition brought by the immune environment, and further weakens the anti-immune response of tumors, which provides strategic significance for the development of cancer immunotherapy in the future.

6. Conclusion

CRISPR-cas9 is a highly personalized therapeutic tool that can not only accurately edit the human genome and provide the basis for the expansion of the human gene pool, but it can also soon be applied to the cancer immunotherapy market. In oncolytic virotherapy, the application of CRISPR/cas9 gene editing offers multiple selectivity for different kinds of oncolytic viruses, enhancing the antitumor ability of the immune system. Knocking out P53 to prevent genetic mutation is a representative example of testing CRRISPR-cas9 utility. In checkpoint immunotherapy, the application of CRISPR-cas9 is to eliminate or suppress some specific genes, like PD-1, to improve the immune responses against tumors. In CAR-T cell therapy and microphage-based immunotherapy, the application of CRISPR-Cas9 is shown in cutting and knocking down inhibitory cytokines of primary T cells and macrophages, achieving to increase the cytotoxicity and capacity for self-healing of host immune cells., respectively. Although it is currently affected by factors such as biocompatibility and off-target effects, with the continuous development of CRISPR-Cas9 technology in cancer immunotherapy potential, it will certainly make more contributions to cancer immunotherapy in the future.

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