

Potential Application of CRISPR Technology in the Development of Immune Checkpoint Inhibitors

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Abstract: Cancer has already become one of the major factors impacting the life expectancy of humans. From long ago, treatments for different types of cancer have been developed in many ways. Among many technologies applied to cancer treatment, clustered regularly interspaced short palindromic repeats (CRISPR) gene editing and Immune checkpoint inhibitor (ICI) are two popular technologies. Currently, there have already been mature treatments for some of the cancers based on these two technologies. Many studies have been conducted to develop new treatments based on CRISPR or ICI, and there are also many ongoing studies in the meantime. However, the potential combination of the two technologies has not been noticed as much as the technologies by themselves. This essay provides a basic description of CRISPR gene editing and ICIs as cancer treatment and discusses the potential combination of the two technologies in the field of cancer immunology and the challenges researchers might have to face during the development of new treatments.

Keywords: CRISPR, checkpoint inhibitors, combination, cancer.

1. Introduction

The research on cancer treatments is never an easy job. Researchers often spend years just to find a substance that might suppress the tumor, and spend more years designing and conducting experiments about it. Though the application of CRISPR technology began two decades ago, and the development of the entire CRISPR-associated nuclease (CRISPR-Cas) system also began approximately the same time, there are no more than 20 types of CRISPR-Cas system [1]. Not only because of the difficulties of studying a new field of cancer biology but also because most of the obvious methods had been tested already. Therefore, to gain new inspiration, it is important to examine the combination of different technologies during the study. For example, in the study of cancer treatments, the combination of ICI technology and CRISPR technology produces various hypotheses about new ICI drugs.

ICI as a cancer treatment, though only widely developed in the last decade, has a relatively long history of discovery. The earliest study on the field of immune checkpoint (IC) occurred in the early 1900s when the effect of IC was first observed. The real discovery and development of ICIs began in the late 20th century, when the most widely targeted IC molecules such as CTLA4 or PD-1/L1 were identified, and the development of drugs that inhibit these molecules was put on the schedule [2]. The true sign of the beginning of the massive innovations of ICIs is the first FDA approval of the ICI drug,

an anti-PD-1 drug named nivolumab in 2014 [3]. Ever since the approval was made by the FDA, the development of new ICI drugs flourished until today.

Compared to the history of ICIs, CRISPR technology is much younger. The first discovery of such DNA sequence occurred in 1987 [4]., and most research has been done on it throughout the entire late 20th century. Besides its discovery time, the application of CRISPR-related technology began early in 2005, although it was not in the field of medicine or pharmacy, but in the production of yogurt [5]. Later in 2012, one of the most important CRISPR technologies, CRISPR-Cas9 was developed by Jennifer Doudna and Emmanuelle Charpentier who won the Nobel Prize in Chemistry in 2020 for such contribution to modern science development [6]. The ongoing study on CRISPR technology is producing valuable results today, and more and more potential applications are on their way to be discovered.

The main construction of this essay will be the discussion of a possible association between CRISPR technology (specifically CRISPR-Cas9) and ICIs. The purpose of this essay is to review CRISPR and ICI technology in order to discover new possibilities of the two technologies, seeking new inspiration that could lead to the combination of the two technologies and the development of new experimental treatments.

2. Structure and Function of CRISPR

The basic structure of the CRISPR sequence can be generally described as a gene sequence with two sections: The first section is the Cas genes, which will vary, depending on the specific type of the CRISPR technology (such as CRISPR-Cas9 or CRISPR-Cas12a) or in natural CRISPR-Cas system exist in bacterias, and function differently; the second section is the CRISPR array, which exists in all types of CRISPR sequences, including the natural ones, that contains the spacers and the repeats. The repeats are 30 base pairs long DNA segments all with the same sequence. They also stand for the “P” in CRISPR - palindromic, because they usually contain a stretch of bases connected to the same bases in a reversed order (root from word palindrome in language - a word or phrase that is the same reading from both the start and the end like “tenet”) [7]. The other structure, the spacers, are small, non-repeating segments that exist between each repeat. One notable fact about the repeats is that they don’t exist in the Cas9 system, because their CRISPR RNA (crRNA) base-pair with trans-activating crRNA (tracrRNA) [7].

The different types of CRISPR-Cas systems have different duties. The most basic, original forms of CRISPR-Cas systems, the ones that exist within natural bacteria, are only responsible for defending the bacteria from phage infection [7]. Once these sequences are found by the researchers, they are used in applications that differ from what they originally did. From yogurt production [5]. to cancer treatment, the CRISPR sequences and different types of CRISPR technologies have been applied in many fields. However, they all achieve their goal in similar ways, because they all function the similar ways. The first step of most CRISPR-Cas systems is the spacer acquisition, where the spacers are formed. This is the process when the target DNA (in natural CRISPR normally the viral DNA from the phages) is captured by the Cas1-Cas2 complex (combination of Cas1 protein and Cas2 protein) and transferred into a new spacer placed at the end of the CRISPR array [8]. Then, the second step of these CRISPR-Cas systems is the crRNA biogenesis. During this step, the CRISPR array, which is a DNA sequence, is transcribed into a long RNA sequence, then separated into small pieces with only one crRNA and one spacer, contained in Cas proteins [7]. The final step of most CRISPR systems is the gene knock-out, or to call it interference. In the field of cancer biology, it would typically be the gene that controls the replication of tumor cells if the CRISPR-Cas system is one of the systems that are created for cancer treatments. However, the target sequence could be any gene depending on the application scenario, since the CRISPR-Cas system is a gene-editing tool.

3. Function of ICIs

ICs play a crucial role in the process of self-tolerance since they prevent the immune system from attacking body cells and organs. However, the reason why researchers would develop all of the existing ICIs is that the function of ICs can be used by some cancers to protect themselves from the immune system by stimulating the ICs [9]. Therefore, the way checkpoint inhibitors treat cancer is to suppress the blockade of ICs and allow the immune system to do its natural job.

There are several types of IC molecules, and most of the ICIs targetting Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), Programmed Death 1 receptor (PD-1), and Programmed Death-Ligand 1 (PD-L1).

CTLA-4 is predominantly expressed in T cells and inhibits T cell activation. They will strongly bond with the CD80 and CD86 ligands on the surface of dendritic cells (DCs), thereby preventing them from interacting with CD28 receptors on the surface of T cells, which would trigger the T cell activation against the DC if occurred [10]. The ICIs targetting CTLA-4 would suppress its function, blocking the interaction between CTLA-4 molecule on T cells and CD80/86 ligands on DCs, allowing the T cells to activate. One notable fact about the application of anti-CTLA-4 drugs is that a lower dose injection in the tumor area has the same effect as a higher dose injected in the bloodstream, which means the concentration of antibodies within the patient is lower. Such changes might result in fewer adverse events that are caused by the immune system due to the absence of the CTLA-4 checkpoint molecule in T cell activity [11]. According to a study published by Luuk van Hooren et al. in 2016, when low-dose peritumoral anti-CTLA-4 therapy is applied to mice with murine bladder 49 (MB49) tumor, the lowest observed effect level was 1.2mg/kg, which is lower than the relatively higher dose for systematic therapy (>4 mg/kg for mice and 3-10 mg/kg for human) [11]. They have also concluded that the tumor-specific memory which prevents the relapse of the same tumor will not be influenced by the administration route of earlier treatment through rechallenging the survived animals and naive animals with Lewis Lung Carcinoma-1 (LLC-1) cells and MB49 tumor cells and record the tumors' growth within both types of animals [11].

PD-1, while CTLA-4 only predominantly exists on T cells, exists in many immune system members including macrophages, B lymphocytes, DCs, natural killer cells, etc [12]. It functions by suppressing the inflammatory activity of T cells, which prevents autoimmune diseases but also reduces the T cells' ability to attack tumor cells [13]. Therefore, ICIs suppressing its function could release the immune system's ability to attack cancer. The reason why it became a major target of ICIs is that one of its ligands, PD-L1, is frequently expressed in several cancers [14], so targetting PD-1 could effectively treat these cancers, such as melanoma and Non-Small-Cell Lung Cancer (NSCLC). The tumor cells would develop PD-L1 ligands on their surface, and interact with the PD-1 receptors on T cells to avoid being attacked. The PD-1/PD-L1 antibodies would bond with all the PD-1 receptors and PD-L1 ligands on both T cells and tumor cells, blocking the interaction and allowing the attack to happen [15]. The effect of PD-1/PD-L1 inhibitors over melanoma and NSCLC is evidenced by the five drugs represented by nivolumab and more than 30 FDA approvals of these drugs as treatment of melanoma and NSCLC in the past decade [3].

Though allowing the immune system to attack the tumor cells sounds very safe and effective, a common issue with the checkpoint inhibitor drugs is the autoimmune diseases they might cause, or to say the harm to normal cells and organs due to the absence of IC's functioning. More related discussion about issues of checkpoint inhibitors will be in the later section

4. Combination Application & Hypothesis

Despite there are many difficulties in combining the two types of technologies, some progress has already been made. Jaiwoo Lee et al., show an example of the combination of two types of

technologies. They applied CRISPR-Cas9 gene editing towards hydrogel to create hydrogels that contain DNA with PD-1 aptamer and sgRNA-targeting sequence, which helps the aptamer to locate the target protein [16]. Another study published in 2020 also applied CRISPR-Cas9 and PD-1 ICI at the same time. In this study, the data collected by researchers shows that CRISPR-Cas9-Associated targeted therapy towards HPV16 E6/E7 could induce cervical cancer cell apoptosis, and the lymphocyte dysfunction is also caused by the overexpression of PD-L1 induced by HPV16 E6/E7, thus combining the targeted therapy with ICIs might produce synergistic effects in treating cervical cancer. Later their experiment on humanized mice containing cervical tumors also provided evidence proving that the combination of gRNA-PD-1 (can be viewed as ICI) with gRNA-HPV16 E6/E7 treatment improved the survival of mice and suppressed the tumor cells effectively [17].

Besides these pre-existing results, untested hypotheses could better help in inspiring ideas about new experiments and treatments. For example, through the definition of ICI and its functions, it is not hard to notice that the ICI treatments are harming normal cells and organs while exposing the tumor cells to the immune system. Then, it is possible to work inversely, suppress the tumor cell genes that allow them to stimulate the ICs instead of suppress the ICs through CRISPR gene-editing, knock out these genes instead of the common target of CRISPR gene-editing in cancer treatment, the gene sequence that controls the regeneration of tumor cells. If this hypothesis is tested and meets the expectations, it will allow the immune system to attack the tumor cells without letting them attack other organs and cause autoimmune diseases

5. Challenges & Issues

Though the prospect of the two technologies seems glorious, there are several problems remaining for both of the technologies and their combination. For the CRISPR system, there are several main challenges left for researchers to solve. The first one is an off-target effect, where the issue is simply the literal meaning of its name, that the sgRNA sometimes misses the target sequence and matches with another non-target sequence, causing unexpected mutations [17]. Though there have been many approaches to solving this problem, it remains a major challenge for the development of CRISPR technology. Another major challenge for the development of CRISPR technology is the immunogenicity of Cas proteins and genes. Since the CRISPR sequences are derived from bacteria, the delivery of the CRISPR-Cas component into the body would trigger the immune system's response, which would obstruct the CRISPR-Cas protein from being effective [18]. On the other side, there are also several challenges for the development of ICIs today. For example, as ICI is also a type of immunotherapy, the resistance to immunotherapy is one of the challenges researchers are facing. There are mainly two types of resistance, primary and acquired: primary resistance refers to the new treatment that is not effective for the cancer in a certain patient, and acquired resistance refers to the drug that used to be successful and no longer works anymore [19]. In both conditions, the effect of ICI is reduced, thus the treatment can no longer inhibit the tumor as well as expected.

Moreover, the combination of the two technologies is also facing challenges. The biggest problem with the combination is unknown. Although the effects of technologies employed separately are already discovered, it is still questionable what will happen if the two technologies are combined. Some approaches have been made, indeed, but there are many possible ways of combination, and most have not been tested yet. The combination of technologies might result in new, effective treatment, but also might result in harsh problems that affect the survival rate of patients. Also, there are still many potential applications of CRISPR technology and ICIs by themselves, so there isn't much necessity for one to examine the combination of the two for the development of new treatments. Therefore, it is reasonable to assume that more attentions are put into developing new treatments constructed by a single technology and solving their pre-existing issues, instead of discovering the possibilities of the combination.

6. Conclusion

In conclusion, CRISPR gene editing and ICIs are well-developed technologies that can be applied to cancer treatment. On one hand, the combination of the two technologies might inspire the development of new treatments; on the other hand, there are still many challenges and issues with the combination of the two technologies. Despite the challenges researchers might have to face, there is still the necessity to try to combine the two technologies and test the treatments that result from the combination of the two technologies. Though the final treatment might be simply the usage of another type of ICI drug, the inspiration that led to the development of the drug might come from the hypothesis about the combination of ICI and CRISPR technology.

Unfortunately, due to the lack of research resources, this essay failed to include more specific details about CRISPR and ICIs and also failed to collect data that supports the hypothesis that came up in the essay. Most content of this essay is based on pre-existing study results and the review papers published earlier, thus does not have any content that is new to the field of cancer immunology. Also, there are many more challenges and issues, for both the technologies themselves and their combination, that are not mentioned in this essay. There could also be many more hypotheses about the combination, yet none were included here except the one mentioned earlier. Lastly, if further research on CRISPR or ICIs starts to slow down, it is feasible for the researchers to turn to a new direction of technology combination, which might open an entire new period of development.

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