Perspectives of gene editing for cancer treatments, in addition to casual cancer therapies

Shuhao Cao[1, a, *, †], Sang Yu[2, b, *, †], Yufei Li[3, c, *, †]

[1]Malvern College Chengdu Grade 12 Chengdu, China

[2]Indiana University Bloomington Senior Tangshan, China

[3]Mercer Island high school Senior Seattle, United States

[*]Corresponding author: [a]Kaden.Cao@malvernchengdu.cn, [b]sangyu@iu.edu, [c]Borisyli@outlook.com

[†]These authors contributed equally

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Abstract: Nowadays, a surge in the number of human diseases has been marked treatable with advanced medical technologies. However, there is still a considerable number of diseases that today's medical level cannot heal. Cancer, one of the diseases that can hardly be treated, has received considerable attention. Cancer is a genetic disease caused by the uncontrolled growth of abnormal cells in the body, which can be caused when normal control mechanisms of the body stop working. This paper aims to introduce the newly developing technology – gene editing (CRISPR is one of the main ideas). In the recent 30 years, CRISPR was investigated and quickly developed, which provides the idea of treating cancer by modifying DNA sequences. However, this technology is not mature enough, requiring further research and experiments to determine whether CRISPR can be used safely on human genetic diseases. The paper provides a brief background of the cancer mechanisms and introduces current treatments such as Chemanol therapy, immunotherapy, radiotherapy, and hormonal therapy with targeted medicine.

1. Introduction

Cancer is a genetic disease in which some of the cells in the human body start to grow uncontrollably and start to spread to other parts of the body. Normally, a cell is multiplied or replicated through the process called cell division, and when old cells die or get damaged, new ones take their place. But sometimes the damaged or dead cells are not replaced and they keep replicating themselves, creating tumors that can then spread to other places in the body creating new tumors. The tumors can be deadly because they do not follow signals sent by the body. For example, a normal cell will only divide when they are signaled to grow, or die when they are signaled to do so, but cancer cells will grow even without such signals and ignore apoptosis, which is a way the body gets rid of unwanted cells. The cancer cells can grow uncontrollably throughout the body interrupting the growth of normal cells and taking away their nutrition, effectively killing them, causing various problems in the human body. Cancer can be caused by many things, like errors that happen when cells divide, damage to the DNA when harmful substances interact with the human body like smoking tobacco or radiation from chemicals or the sun, or it can be inherited from parents. As the body ages the probability of getting cancer increases. Normally, the body is able to detect defective cells and eliminate them before they can spread, but as the body gets older, its ability to do so decreases making cancer more likely to occur. Also, since cancer is a genetic disease and everyone's genetics are different, some people will just be more prone to getting cancer.

There are many kinds of cancers, and they are breast cancer, lung and bronchus cancer, prostate cancer, colon and rectum cancer, melanoma of the skin, bladder cancer, non-Hodgkin lymphoma, kidney and renal pelvis cancer, endometrial cancer, leukemia, pancreatic cancer, thyroid cancer, and liver cancer. Out of those, lung and colorectal are the most common amongst both men and women.

On average, 442 out of 100000 men and women get cancer each year, and the death rate of cancer is about 158 out of 100000 while women have a lower mortality rate than men.

Cancer treatments are various, including chemotherapy, which uses drugs to kill the cancer cells, hormone therapy, which stops the growth of prostate and breast cancer, immune therapy, which helps your immune system fight cancer, radiation therapy, which uses radiation to kill cancer cells, and normal surgery that removes the tumor in the body. Out of all the treatment options, chemotherapy is the most common treatment used. It can be used to treat almost all types of cancer and have a good effect in preventing tumors from growing.

2. Theoretical understanding

2.1 Gene & cancer

Cancer is the group of abnormal cell replication that can invade and separate other parts of the body. One of its potential mechanisms is closely related to CDKs (Cyclin-dependent kinases) and cyclin. CDKs are the series of proteins that control and promote the cell cycle continuously, it prevents the cells that are carrying mutated genes pass through the cell cycle and replicating. There are 4 CDKs 'check points' in the cell cycle. The first checkpoint is at end of the G1 phase to check the DNA sequence. The second checkpoint is at end of the G2 phase which makes sure cells are no problem entering the mitosis. When cells are at the point of going to the next stage, a specific cyclin will combine with CDKs to release a transcription factor to allow that cell through the next phase (eg. E2F). Excessive CDKs will make the cells continuously enter the cell cycle which makes the cell uncontrollably grow, and finally lead to cancer. Genetic mutation is the main cause of the increase in the number of CDKs.[10] There are a few types of genetic mutations on DNA, including point mutation, DNA amplification, chromosomal rearrangement (chromosomes connect where they should not.). Epigenetic modifications are also important, Methylation and Acetylation of DNA can silent some genes or make them more active. Uncontrolled growth is caused by two main changes:

1. Activation of Oncogenes (eg. Myc and ras).
2. Inactivation of tumor suppressor genes (eg. P53) [11].

Oncogene's activation, using the RAS gene as an example. RAS gene is the sequence that can produce RAS protein which is an intercellular protein below the plasma membrane. There is a growth receptor beside the RAS protein. Normally, the growth factor will stimulate the growth factor receptor, then active RAS protein. Activated RAS protein will go through a cascade of inter-cellular phosphorylation which essentially activates transcription factors. Once they are activated, they will go to DNA to produce CDKs and cyclin (at end of the G1 phase). Therefore, if there is a mutation happened on the RAS gene, the cell will overproduce CDKs and cyclins for cell growth. Meanwhile, Myc genes can be transcript to trigger cell growth and activity. If mutation happened on that gene which will also cause uncontrolled cell growth.

On the other hand, P53 protein, which was originally used to activate P21 protein to arrest the cell, and also repair the DNA, if those did not work, P53 will also make important protein for apoptosis of the cell. If mutation happened to stop the production of P53, those things cannot happen. Finally, the cell wall by the path through the checkpoints and continuously grow and proliferate [11].

The genes play a very important role in developing cancer or metastatic nodules, presently, most approaches are for monogenic gene therapy, tackling one or more critical gene defects. Gene engineering is the main field of gene therapy, especially on genetic manipulation to directly modify the genome in a cell. There are 3 main methods of genetic manipulation.

1. Plasmid method
2. Vector method
3. Biolistic method

Cancer has different categories, some of them are named by original infected tissue (primary tumor, such as lung cancer), some of them are named by infected cell or tissue such as Sarcoma, Myeloma. Tissues with many cell divisions are more likely to develop tumors. Therefore, squamous cell

carcinoma (squamous cell carcinoma), adenocarcinoma, large cell carcinoma, small cell carcinoma, etc. are all classifications of malignant tumors based on different histopathology. [12] Meanwhile, in the same type of cancer, cancer is usually subdivided according to the following conditions. In the same type of cancer, these factors together guide the treatment of cancer patients. The more accurate the classification, the more likely it is to benefit from the treatment of cancer.

Tumor grade*:* grade is a measure of tumor aggressiveness. Grade I tumors are less aggressive, and the cells may be very similar to normal cells at the beginning of cancer. In contrast, grade III tumors are usually more aggressive, and the cells look very different from normal cells.

Tumor staging*:* Tumors are staged in different ways, but many tumors are staged between 1 (stage I) and 4 (stage IV), of which stage 4 is the most advanced stage of cancer. For the same cancer type, treatment methods (combinations) are different for each stage.

DNA/molecular profile: As our understanding of genetics improves, tumors are classified more and more frequently in terms of genetic profiles. For example, some lung cancers have EGFR mutations, while others have ALK rearrangements. For the same cancer type, the molecular profile is different, and the treatment and prognosis are also different.

2.2 Gene editing and CRISPR

The term “CRISPR” can be defined as clustered regularly interspaced short palindromic repeats. “CRISPR” is an abbreviated form of the “CRISPR/Cas” system, the only adaptive immune system found in prokaryotes without any other comparable system. It is commonly found in archaea and bacteria. “Cas,” in the term of “CRISPR/Cas” system, means “CRISPR-associated” proteins. Based on the area and function, CRISPR/Cas system can be classified into two main classes: class 1 represented as “multi-subunit effector complex,” which means the module is composed of a multi-protein complex, including type I (Cas3), type III (Cas 10), and type IV (Csf1); class 2 represents as “simple multi-domain effector,” which means only one effector protein is required, including type II (Cas9), type V (Cpf1), and type VI (Cas 10). In those sub-types, the type II system is the one that is worth studying most. In the type II CRISPR system, the specific CRISPR-associated protein is Cas9, the enzyme needed to cut strands of DNA. Because of the specific function of Cas9, CRISPR-Cas9 is considered a powerful technology for changing the expression of a gene by cutting and editing specific stretches of DNA. CRISPR technology was first described as a gene modification tool by Jennifer Doudna, a biochemist from the University of California, Berkeley, and Emmanuelle Charpentier from French. However, CRISPR was not used in the molecular biology laboratory. According to Jennifer Doudna, researchers who investigated CRISPR first were food scientists. Scientists used the technology to prevent the Streptococcus strains from infecting the virus to make dairy products, such as cheese and yogurts. By that time, the name “CRISPR” was not even defined. In 1987, the first case of CRISPR was found in the E.coli genome. Yoshizumi Ishino, a professor from Osaka University in Japan, was the first one to detect the distinct nucleotide repeats and spacer in the E.coli microbe. At that time, because of the lack of DNA sequence data, those repeated sequences were difficult to predict their biological function. The first case of CRISPR found in archaea was observed in 1993, while the technology for genetic analysis improved. After then, more CRISPR cases were detected in both bacterial and archaeal genomes. In 2002, the term “CRISPR” was published by Ruud Jansen from Utrecht University in the journal “Molecular Microbiology.” In 2012, a research paper in journals “Science” and “PNAS” described that CRISPR-Cas9 can cut DNA in a specific area, which makes the neutral CRISPR system considered a powerful gene-editing tool.

To understand the mechanism of CRISPR, a few terms need to be introduced. The first term is DNA, an abbreviated form of deoxyribonucleic acid used to store genetic information for most organisms. The other term, “RNA,” is ribonucleic acid, a molecule with multiple functions, such as transporting DNA and DNA transcription. One type of RNA that plays an essential role in the CRISPR system is the Guide RNA, usually shown as “gRNA.” In the CRISPR system, gRNA is the molecule that binds to the Cas9 enzyme. Based on the sequence of gRNA, the location of Cas9 that cuts DNA can be easily determined. The mechanism of the CRISPR system can be easily understood after knowing previous terms. The Cas9 protein is used to chop up DNA at a specific sequence, which a

guide RNA determines the location. Generally, the Cas9 enzyme will cause double-stranded breaks on DNA, leading to either of two repair mechanisms: one is non-homologous end joining, and the other one is homology-directed repair. In non-homologous end joining, the repairing of breaks is pulling two ends together without any additional template. It is a much better method when knocking out a gene is necessary. However, it is hardly possible to match those two ends perfectly. In this case, the high risk of target gene interruption and disabling is one of the significant concerns. The other kind of repair, homology-directed repair, is more useful when inserting is desired. In this method, the missing sequences can be filled with a template that contains its copy of DNA. In this way, the cell can be modified using any templates; it can be manipulated from a small mutation to a different gene.

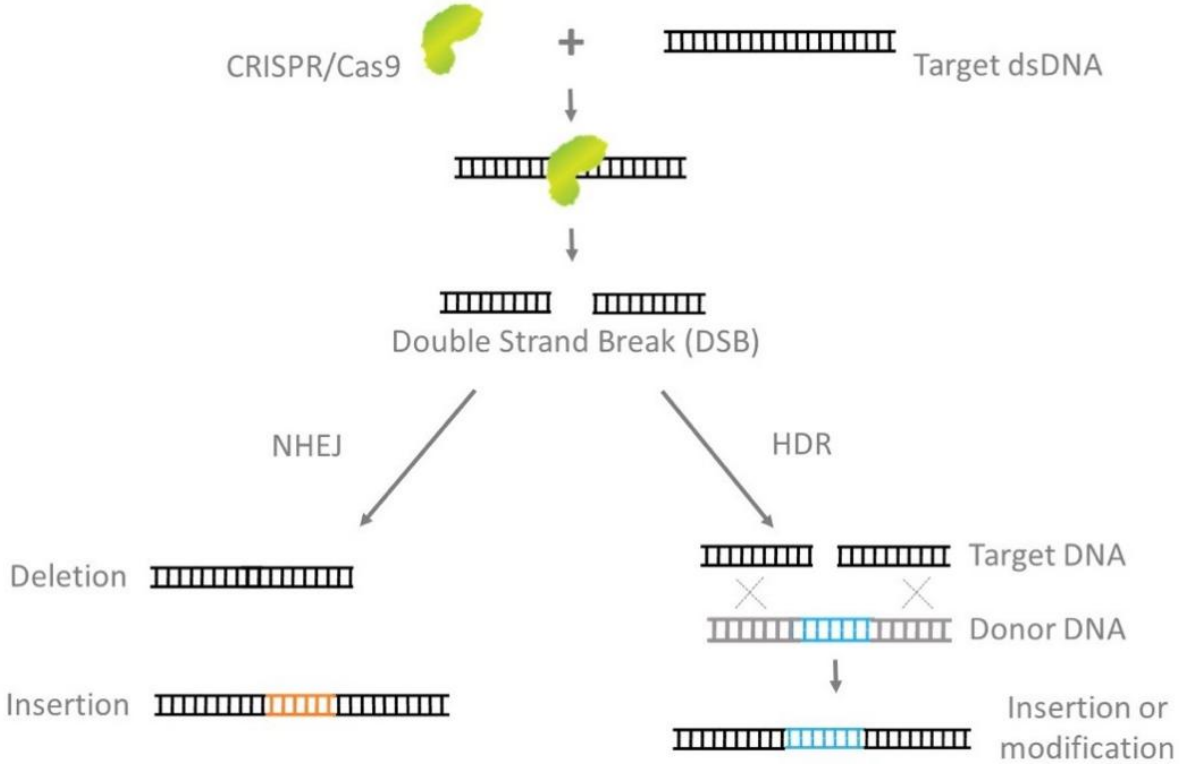

Figure 1. The figure above shows two repair mechanisms by using the CRISPR-Cas system. Cas9 enzyme causes double-strand break (DSB). In this figure, NHEJ represents non-homologous end joining, in which deletion and insertion often occur. HDR represents homology-directed repair, in which insertion and modification often occur. (The graph is from: https: // www.goldbio.com/articles/article/CRISPR-discovery-and-adaptation).

Even though CRISPR technology has been developed rapidly in the recent 30 years and has shown its significant advantages compared to the previous gene-editing methods, it is still not mature enough to use in most human diseases. According to Blake Wiedenheft of Montana State University in Bozeman, the repair enzyme may have a chance of not following the template, which causes it to attach back to DNA and does not finish its desired changes. Therefore, many types of cells have no successful case of template editing. Another big concern of CRISPR use in human disease is that the Cas9 enzyme may accidentally cut a bit of DNA that is not accurate enough. Ever since the DNA breaks, there is the possibility to cause cancer because chromosomes will rearrange. In a word, more accurate improvement is needed for treating more human diseases.

The current application shows that CRISPR technology can be used as a treatment for lung cancer. There are two different ways. One way is to slow cancer progression by creating single directed RNA and Cas9 protein, which can distribute to cancer cells, the other way is to control the expression of ligand-receptors, which can also slow the cancer progression by reducing the connection between the tumor cell and its ligand-receptors. This application is not an only achievement in treating human cancers but also a key shows the bright future of CRISPR technology. The method of CRISPR provides a distinct way to treat cancer by modifying the genome of the target cells. Even though CRISPR is still

improving; its efficiency, convenience, and character of simple and adaptable would make CRISPR a top choice of cancer care.

3. Applied cancer therapies

With the presence of lots of treatments, different treatments will depend on the stage and type of cancer.

Chemotherapy is the most common therapy to use when tumors remain localized at the time of diagnosis about one-third of patients are cured with local treatment strategies, such as surgery or chemotherapy. Chemotherapy has 3 main clinical setting

1. Primary induction: when cancer is not sensitive to other existing treatments at all. The main aim for it is to relieve tumor-related symptoms, improve the overall quality of life, and prolong time to tumor progression

2. Neoadjuvant treatment: for treating localized cancer which showed less effectiveness for existing treatment to s to reduce the size of the primary tumor so that surgical resection can be made easier and more effective eg. Surgery.

3. Adjuvant treatment: one of the most important roles of chemotherapy, which is applied after surgery. The aim for it is to reduce the incidence of both local and systemic recurrence and to improve the overall survival of patients.

3.1 Chemotherapy

The method of using a combination of different drugs to eliminate abnormal cells in different tissues. Therefore, the use of drugs including type and dosage is extremely important. First, it determines the maximum number of cell kill as long as the host can compromise within the range of toxicity. Second, it provides a broad range of interactions between drugs and tumor cells. Finally, it slows down the subsequent drug resistance of cells.

There are several drawbacks of using chemotherapy. Because chemotherapy kills every cell in a specific area, the level of leucocyte will have a significant drop which triggers lots of side-effects and sequela. Those symptoms of sequela including hair loss, weight loss, and lack of blood supply, etc will strongly affect the quality of life of patients. Also, it may develop drug resistance, so the combination of drugs may not work at the same level of effectiveness. Before you begin to format your paper, first write and save the content as a separate text file. Keep your text and graphic files separate until after the text has been formatted and styled. Do not use hard tabs, and limit the use of hard returns to only one return at the end of a paragraph. Do not add any kind of pagination anywhere in the paper. Do not number text heads-the template will do that for you?

3.2 Radiotherapy

Another common use treatment for cancer is radiotherapy. The main principle of radiotherapy is using high-energy radiation to damage the structure of the genetic material of the cell (DNA). Even though the radiation will affect both normal and cancer cell, during treatment will try to maximize the effect on cancer cells minimize the effect on normal cells besides the cancer tissue or on the route of radiation. The previous paper suggests that normal cells tend to repair faster than cancer cells and function normally afterward. [20][21]

Radiotherapy is normally used with a combination of other treatment and perform different role and effect in each cure. For example, if using radiotherapy before surgery the aim is to make the size of the tumor smaller but using it after surgery the aim change to microscopic tumor cells that may have been left behind.[22][23]

Radiotherapy has two main ways to remove cancer cells, rather indirect or direct. The indirect route will need free radical to involve in. However, there are lots of different techniques based on different biological properties and conditions of patients eg. Fractionation, 3DCRT, IMRT, SBRT.

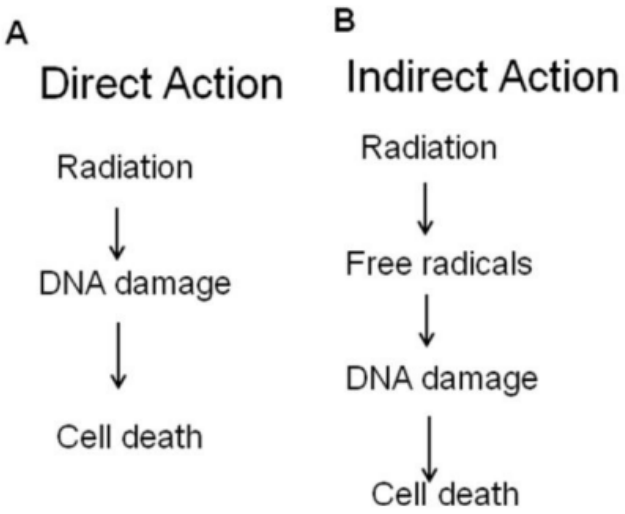

Figure 2. The two main routes of radiotherapy leads to cell death. [22].

Meanwhile, because the different technologies can be used to cure advanced cancer, the route of cell death differs. In addition, the side effects of radiotherapy are very similar to the side effects of chemotherapy, so patient life quality after treatment is still a huge indicator for successful treatment.

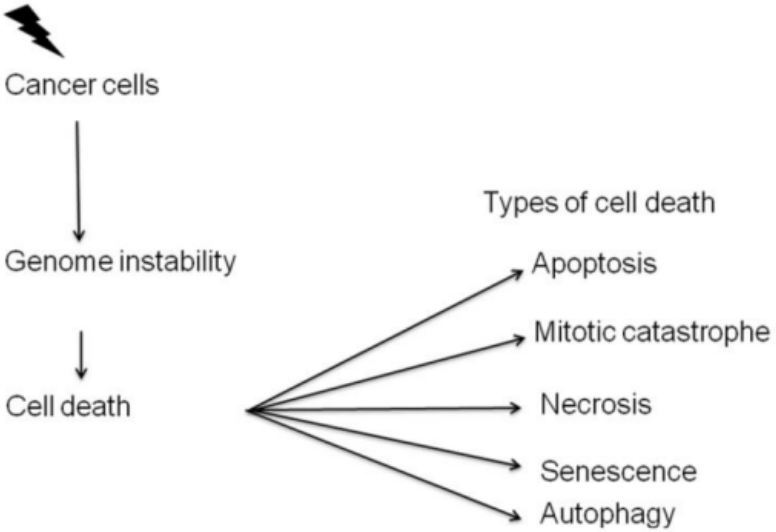

Figure 3. Types of cell death induced by radiation. [22].

Thus, radiation remains an important modality for cancer treatment with ongoing efforts towards designing new radiation treatment modalities and techniques which continue to improve the survival and quality of life of cancer patients.

3.3 Immunotherapy

The recent new technique of cancer therapy, this method is based on the ability of the T-cell which is the most powerful immune cell to eliminate cancer cells. This therapy are mentioned and confirmed in The US Food and Drug Administration (FDA) approval in 2017 and also proved by the United Kingdom and Canada. [24]

There are different types of T-cell technique, adaptive cell therapy attracts most researchers' attention, and the method of using this therapy is similar to the way of how people grow artificial insulin in bacteria. By removing T cells easily collected from patient's blood to virus or modified bacteria, it will expand in vitro to large numbers, and re-infused back into the patient to eradicate tumors. To start with this strategy, tumor-infiltrating lymphocytes (TILs) were extracted from tumors and the infusion of this TIL will make a long-term clinical remission to the patients.

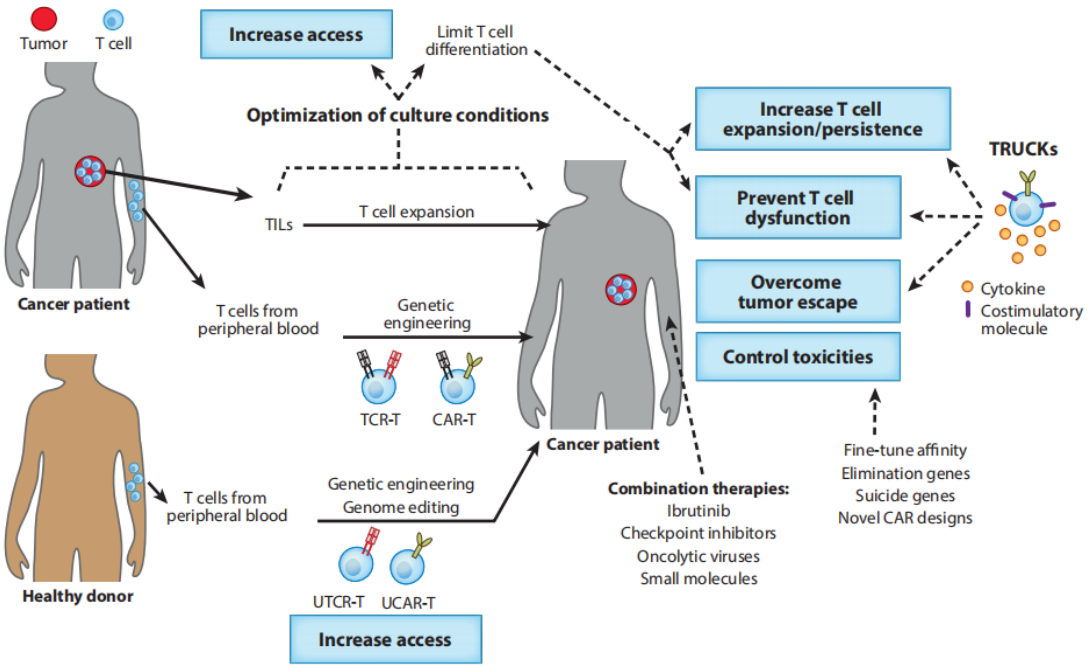

Figure 4. The process of Adoptive T cell therapies. Peripheral T cells have been genetically modified to form Tumor-specific T cells which can survive in the microenvironment of tumors to eliminate tumor cells [24].

Nowadays development in genetic engineering and human biology are given out the possibility of artificially designed T cell injection. This method includes edition in T-cell receptors such as TCR and CARs, which can target the specific tumor cell. The best clinical results with ACT have been obtained mostly with CAR-T cells for CD19+ leukemia and lymphoma which is the bio surface marker on B-cell. [24].

The main essential component of CAR includes extracellular targeting tumor cell receptor and intracellular signaling portion comprised of costimulatory and activation domains that initiate processes including activation, clonal expansion, and cell killing [25].

4. Conclusion

In all, this paper summarizes some early history of cancer, provides basic information on gene and immunotherapy, shows some types of cancer and cancer treatments, and ends with some public opinions on cancer and its treatments. With more thorough understanding of tumorigenesis as well as the advanced technologies such as CRISPR summarized here, cancer would be cured with more confidence Om the near future.

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