Tackling Cancer using the method of CRISPR

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DOI: 10.29322/IJSRP.10.01.2020.p9712
http://dx.doi.org/10.29322/IJSRP.10.01.2020.p9712

Abstract- Over 500,000 people in the United States and over 8 million people worldwide are dying every year from cancer. As people live longer, the incidence of cancer is rising worldwide, and the disease is expected to strike over 20 million people annually by 2030. Cancer is a genetic disease; thus, CRISPR is a method enables us to delete a portion of the genome. Other treatments did not serve the wanted demands; that is, it could help in tackling cancer, but with victimizations. Hence, it is time to tackle the Cancer by this genome editing tool.

Index Terms- CRISPR Technology, the combination of guide RNA and Cas9, Genome-engineering, cell-based therapies, versatile tool for genome engineering.

I. Introduction

CRISPR/Cas9 has become an incredible strategy for making changes to the genome of numerous life forms. First found in quite a while as a major aspect of a versatile resistant framework, CRISPR/Cas9 and adjusted adaptations have discovered an across the board use to build genomes and to actuate or to subdue the declaration of qualities. Accordingly, CRISPR/Cas9 vows to quicken malignant growth explore by giving an effective innovation to dismember instruments of tumorigenesis, recognize focuses for tranquilize improvement, and conceivably arm cells for cell-based treatments. Here, we survey ebb and flow utilizations of the CRISPR/Cas9 innovation for malignant growth research and treatment. We portray novel Cas9 variations and how they are utilized in practical genomics to find novel malignant growth explicit vulnerabilities. Moreover, we feature the effect of CRISPR/Cas9 in producing organoid and mouse models of malignant growth. At last, we give a diagram of the main clinical preliminaries that apply CRISPR/Cas9 as a helpful methodology against disease.

II. Cancer

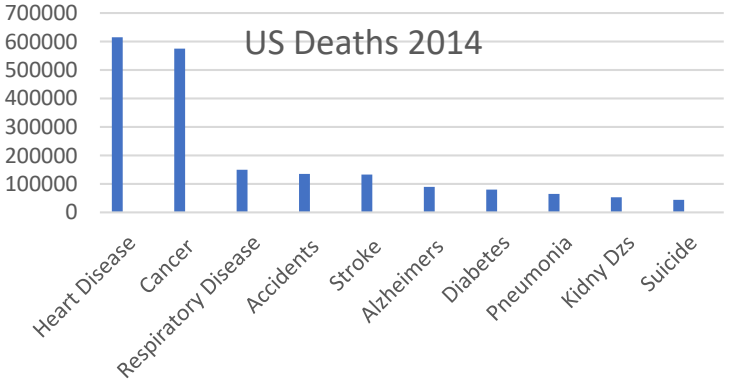
From the scratch, it is much better to identify the problem to be solved well. Hence, "*What is meant by cancer?*" Cancer refers to various terms: uncontrolled growth, tumor, and neoplasm. Nevertheless, Cancer is a disease caused by the uncontrolled division of abnormal cells in a part of the body. In other words, it is a swelling of a part of the body, generally without inflammation, caused by an abnormal growth of tissue, whether benign or malignant. Therefore, Cancer is caused by an accumulation of detrimental variation to the genome. It's important to member that a single mutation is not sufficient to induce cancer formation. The most feasible branch that involve cancer studying and researching is the Oncology. The term oncology literally means a branch of science that deals with tumors and cancers. The word "onco" means bulk, mass, or tumor, while "-logy" means study. Metastasis is the spread of cancer from primary site to distant organ.

III. Cancer Classification

Types of cancer are classified according to the tissue in which they originate to start their attack. There are classified into four main types:

- Sarcoma arise from connective tissue that is found in bones, tendons, cartilage, muscle, and fat.
- Carcinoma arise in epithelial tissue that is found in the internal and external lining of the body.
- Adenocarcinomas develop in an organ or gland.
- Squamous cell carcinomas develop in the squamous epithelium of organs, including the skin, bladder, esophagus, and lung.
- Leukemia is cancer of the blood that originate in bone marrow.
- Lymphoma is cancers of the lymph system.

The first step toward cancer is the Hyperplasia, which is



the primitive growth of the normal cells. After that, the cell goes through the Mild Dysplasia, in which the cells start up to bunch each other with unnormal shapes. As cancer progresses, the cells go through a carcinoma to start the invading trip. According to the statistics and the following graph, cancer is in the second place, after heart attack, for the causes of death in the US (2014): that is, it killed about 575000 US citizen.

Types of Cancer according to the most common worldwide:

1. Lung cancer (13% of all cancers diagnosed; 1.8 million)
2. Breast cancer (12% of all cancers diagnosed; 1.7 million)
3. Colon cancer (10% of all cancers diagnosed; 1.4 million)
4. Prostate cancer (8% of all cancers diagnosed; 1.1 million)
5. Stomach cancer (7% of all cancers diagnosed; 952,000)
6. Liver cancer (6% of all cancers diagnosed; 782,000)

IV. CRISPR–Cas in its prime

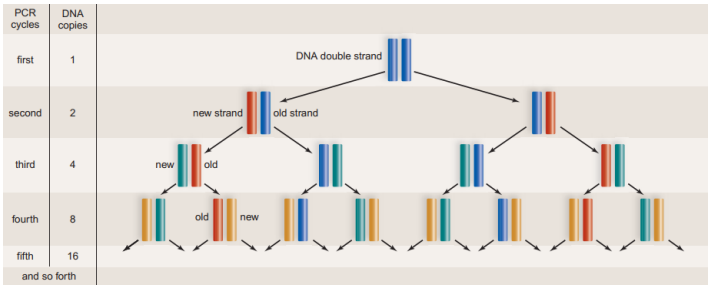
The CRISPR–Cas technology has transformed genome editing by relying on nucleic acid base pairing for target specificity. However, most known human disease-relevant mutations remain difficult to correct owing to the formation of insertions and deletions (indels) upon repair of Cas-generated DNA double-stranded breaks (DSBs) and the low efficiency of introducing precise changes through homology-directed repair in most cell types. Anzalone et al. now introduce CRISPR–Cas9-based 'prime editing', which overcomes these obstacles by relying on reverse transcription for editing.

The nuclease Cas9 is targeted to specific sequences by single guide RNAs (sgRNAs) that hybridize with the target DNA. The authors fused a disabled, 'nickase' form of Cas9 that cannot make DSBs to a reverse transcriptase (RT). In addition, they engineered pegRNAs (prime editing guide RNAs), which are sgRNAs that include a sequence that serves as template for precise editing through reverse transcription. When the Cas9 domain produces a nick, the RT polymerizes a modified DNA segment from the template pegRNA directly into the template DNA strand, forming a heteroduplex with the non-edited strand, which is converted into the edited sequence by DNA repair pathways. Importantly, this prime editing system can accommodate long (≥30bp) RT templates, and thus its targeting is not constrained by the availability of nearby protospacer adjacent motifs. Next, pegRNA optimization and the engineering of mutant RT with improved functionality increased editing efficiency and specificity. These were further increased by augmenting the conversion rate of the non-edited strand in the heteroduplex with the additional use of sgRNAs matching only the edited sequence.

In this way, the non-edited strand is also nicked, but only upon flap-resolution of the edited strand, to minimize concurrent nicking of both strands and thus DSB formation. In human cells, prime editing corrected a transversion causing sickle cell disease, and the 4bp insertion that causes Tay–Sachs disease. The system functioned also in terminally differentiated mouse neurons, and can edit all types of local mutation with high efficiency and low off-targeting levels. Based on the targeting scope and range of insertions and deletions supported by prime editing, it could in theory correct up to ~89% of known human pathogenic mutations.

V. Identify the sequence of cancer exists

The first thing to think in to start the process is how to know the sequence of bases that make the cancerous genes. To know the sequence of bases, whether adenine, thymine, cytosine, or guanine, Polymerase Chain Reaction (PCR) and Electrophoresis should be approached to know the sequence of bases of cancer portion within the genome. The polymerase chain reaction (PCR), developed by Kary Mullis in 1985, can create copies of a segment of DNA quickly in a test tube. PCR is very specific—it amplifies (makes copies of) a targeted DNA sequence. The targeted sequence can be less than one part in a million of the total DNA sample. PCR requires the use of DNA polymerase, the enzyme that carries out DNA replication, and a supply of nucleotides for the new DNA strands. PCR is a chain reaction because the targeted DNA is repeatedly replicated as long as the process continues. The colors in the following figure

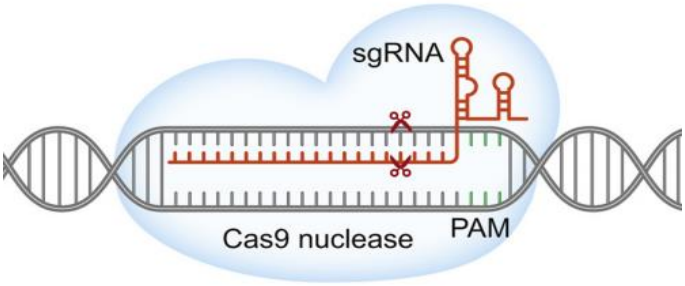


distinguish the old strand from the new DNA strand. Notice that the amount of DNA doubles with each replication cycle. PCR has been in use for years, and now almost every laboratory has automated PCR machines to carry out the procedure. Automation became possible after a temperature insensitive (thermostable) DNA polymerase was extracted from the bacterium Thermus aquaticus, which lives in hot springs. The enzyme can withstand the high temperature used to separate double-stranded DNA; therefore, replication does not have to be interrupted by the need to add more enzyme.

After we have got a copy of the genome portion, we enter it into the electrophoresis mechanism. In electrophoresis, we use the Gel Electrophoresis in plate, with different charged sides, has agarose and gapes in the negative side of the plate. Remembering that the Fragments we have got from PCR is florescent by specified colors. Then we apply these fragments to the electrophoresis to assort according to their size. As DNA has negative charge it repeals from the negative charge to the positive one. Moreover, the shorter fragment, the faster it will attract to the positive pole. At the end of the electrophoresis plate, these is a florescent color reader that lights according to the florescent base passed within it. From the lights, we can figure out the sequence of bases that make this type of cancer in the cell.

VI. Preparation of the Cas9 combination

Consequently, we have accomplished the Guide RNA portion that will be combined with the Cas9 protein to make the CRISPR combination. In contract, we will enter the guide RNA into the Cas9 protein, after being extracted from the cell by

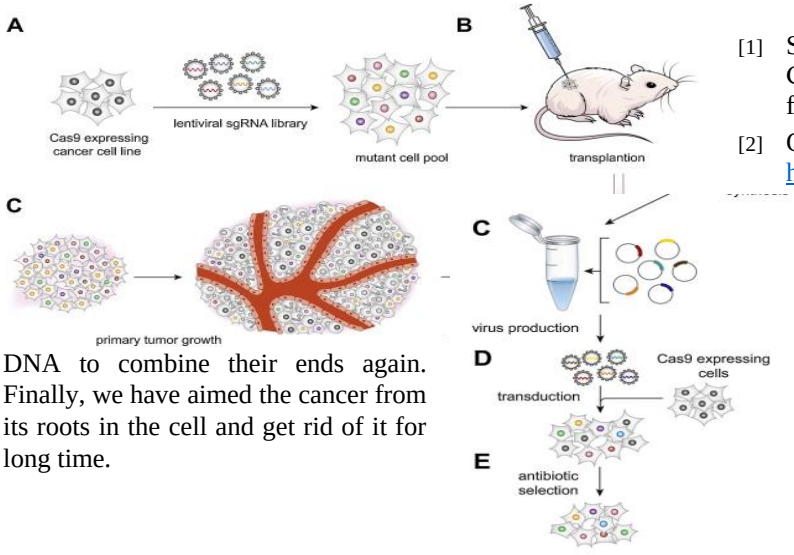


fractionation, by using plasmids as shown in the following figure. Finally, we have got that combination of Cas9/ Guide RNA protein ready to be injected into the targeted tumors to detect cancer.

The combination will enter the cell and each ingredient will do his function; that is, the Cas9 protein, carrying the sgRNA, will attach to the DNA of the cell and start to sperate the double stranded DNA searching for the portion that 100% analogous to the sgRNA inside it.

Cas9 protein work as slasher that slash the DNA quickly once it finds the analogous portion if the DNA according to its sgRNA. Once it finds that portion it slashes the DNA at that part from the end and the start to get rid of that portion which represents the Cancerous bases within the cell's DNA.

After slashing the cancer part of the genes from the whole genome, it is time for ligase enzyme to do its job. Ligase is a catalyst that can catalyze the joining of two enormous particles by framing another substance bond, for the most part with going with hydrolysis of a little pendant concoction bunch on one of the bigger atoms or the chemical catalyzing the connecting together of two mixes; hence, it attaches that sticky ends of the slashed



DNA to combine their ends again. Finally, we have aimed the cancer from its roots in the cell and get rid of it for long time.

VII. Summary

As a whole process, it all revolves around maintaining CRISPR method in dealing with this rooted disease from its origin. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is method designed to engage with DNA sequencing. The whole idea revolves around using Cas9 protein and Guide RNA analogous to the targeted DNA we want to abolish which are the cancer mutations in the cell. This combination of protein and guide RNA induced the cell to scan the DNA till detecting the part of nucleotides similar to the guide RNA. After finding it, the two strands of DNA separates and the one corresponding to the guide RNA attach to it. Then the Cas9 protein receives a sign for slashing the DNA from this part to delete it and to be considered among DNA fragments.

Affording us the advantage of editing the DNA molecules, CRISPR technology is a simple effective tool for editing genomes. It allows scientists to easily alter DNA sequences and modify gene functionality. Its high potential applications include correcting genetic defects, treating and stopping the spread of diseases and enhancing crops that seem harmful or unwanted in the form of mutations or diseases.

VIII. CONCLUSION

In conclusion, CRISPR is an effective technology affords us the opportunity to edit the human genome by the removal of undesirable treats beyond the gene expression. Applying it to cancer is one of the ideas in new lights in medicine and sciences; however, with the study and research, it was expected that it is applicable and have met the achievements. Hence, why not use it? I think that CRISPR is going through considerable growth the coming years-growth will save the world.

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