REPORT: CRISPR/CAS9 GENE EDITING TOOL IN ONCOLOGICAL RESEARCH

INTRODUCTION:

CRISPR/Cas9 is the basis for bonafide adaptive immunity in prokaryotic organisms which offers heritable immunity.[1] This CRISPR/Cas9 system is effective in exact gene corrections, and has been proven to have both accuracy as well as versatility.

Cancer is a genomic disorder that includes alterations, and mutations of various genes, translocation and chromosomal insertions and deletion, all leading to proliferation of cells without regulated apoptosis, and causes tumors and numerous other secondary issues. Cancer has been proven to be fatal, and a search for a potential cure for this versatile disease has become a vital part of medical research these days.

Rewriting a part of the genetic code using CRISPR/Cas9 is blooming to be the new technology, the efficacy of which seems promising for researchers.

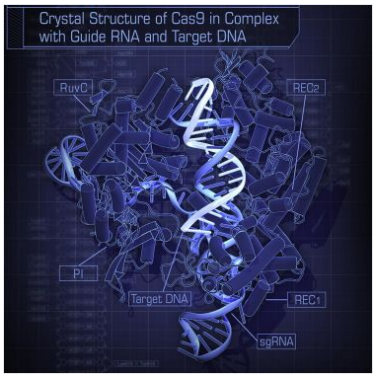
HOW THE CRISPR/CAS9 WORKS:

CRISPR : C - Clustered
R - Regularly
I - Interspaced
S - Short
P - Palindromic
R - Repeats

Is the basis of a genetic defence system bacteria, archaea bacteria and prokaryotes. This mechanism is used when viruses or virophages attack the organism.

The CRISPR/Cas9system has two essential components. The first one is an endonuclease called the Cas9, and the second one is the sgRNA.the sgRNA attaches to the targeted DNA sequence since it is complementary to the target DNA sequence . After the sg RNA is bound, the endonuclease starts the break of Dna in a sequence specific double stranded fashion. Then, the cell employs the DNA repair mechanism, This repair mechanism is of generally two types: non homologous end joining and homology directed pathway. The first pathway is faster but error prone and leads to loss of genetic information. The second pathway despite being slow, is more accurate and effective[2].

During this repair, the DNA is then modified by CRISPR as needed before the repair is done. The final end product of the DNA ends up with insertion , modification or deletion of certain genes. IN bacteria, this usually involves the removal of essential genes in the virus to make those viruses weak and ineffective to attack the organism[3].



[3]

ANALYSIS OF THE CANCER IN THE GENOMIC LEVEL:

Cancer is a multiple hit disease, that is, it is a complex disease with many number of point mutations that together result in many fatal symptoms. Cancer is still a very large cause of death globally and is a mental and physical anguish to the suffer and the family members as well. A need to find the cure for this disease is a necessity with this mortality rate.

Cancer can be caused by various reasons. One of the most common causes is the expression of oncogenes. Oncogenes are the genes that are responsible for the uncontrolled growth of cells, the biggest reason for the fatality rate of cancer[4].

Oncogenesis happens early in cancer. This could be due to the mutations of the pro-oncogenes. It is generally one cell that undergoes this mutation called oncogenetic transformation. This can further lead to metastasis or intruding of cancer into other body parts[5].

At times, viruses like sarcoma virus transfer solid tumors , or these retroviruses insert oncogenes that interfere with the transcription of proto-oncogenes. Sometimes some chemicals called carcinogens can act as mutation initiators too . There is a chance that the cancer tumor starts to grow due to a faulty damaged-DNA-repair-mechanism. Cancer has also been studied to be sometimes caused by damage of tumor suppressor genes, which are supposed to check, modulate and regulate controlled cell growth as well as apoptosis[6].

The causes to cancer are varied, which makes the treatment and cure of this disease a very tricky job. One of the oldest known cases of cancer was in 3000BC where there is a mention of a tumor with no apparent cause for it. Though it wasn't called cancer back then, it is needless to say that scientists of all time have made every effort to find a cure for this deadly disease. Let us try to understand what those treatments are and how and try to understand how they have not been very effective.

KNOWN TREATMENTS FOR CANCER:

Oncogenes can be targeted to treat cancer effectivelyTumor suppressor genes can be reactivated so that they can continue the inhibitory effect on the multiplication of cells. Both of these can be achieved by the use of drugs to either stop, or regulate the expression of the specific genes. This is done in a process called chemotherapy. But these drugs are not always effective, for reasons ranging from some surviving cells muto get resistant againtose drugs to gene amplification or the cancer cells refusing to take in the drug[7].

Some other conventional methods of treating cancer could be surgery, radiation therapy, hormonal therapy. All of these methods have been less than successful in many cases of cancer. Retroviral therapy is another method of treatment that involves retroviruses being used to cure cancer. Here, the retroviruses are subjected to artificial evolution to identify oncogenes, modify or delete a specific sequence of the genome. The potential danger in this therapy leading to even more dangerous off target effects is the reason it is still under extensive study and consideration[6].

MOLECULAR BIOLOGY TREATMENTS FOR CANCER:

ORF, ZFN,TALEN have all been used in the past as molecular biology tools to deal with cancer treatments. Out of these, ZNF or zinc finger nuclease that can cleave sequence specific targets.Later, as the repair mechanism kicks in, the new genome content has the modified genes [8]. An effective treatment seemed to be RNAi. RNA interference involves a small non-coding RNA that binds with functional mRNA's and potentially inhibit the translation of those messenger RNA's. RNAi can target specific genomic sequences and it can inhibit many genes simultaneously. It has also proved to be a useful tool in creating personalised drugs for cancer patients which are more effective in controlling tumor growth. It has a very deterministic outcome in mammalian cells[9].

However studies have proved that RNAi can lead to some unpredicted as well as non-specific toxicity, not to mention its off target effects. It also sometimes has incomplete gene knockout or temporary gene dysfunction that could be potentially dangerous.Thus, the search for an even better gene-editing tool continued.

CRISPR/Cas9 is the most recent weapon of choice to fight cancer . The working of CRISPR is this way, if the cancer causing gene is known, which many scientists over the years have researched and discovered and stored in databases, then the CRISPR/Cas9 system can be introduced into those cells to cut off or introduce new genes. Here, it is possible for the CRISPR/Cas9 system to delete the oncogenes, or remove and replace the regulator genes that can control apoptosis or can introduce a completely new gene that can stop the growth of the tumor.

For example, there are current clinical trials that target the proteins of programmed cell death and programmed cell death ligands , which are necessary to negatively regulate immune cells, by blocking these two proteins, the survival rate of immune cells can substantially increase, and thus increasing survival rate[10].

This tool is more effective for a single gene mutation, but researchers have been digging deeper to expand it to modify multiple genes at the same time by injecting multiple guide RNAs at the same time to produce multiplexed mutations. One obvious advantage of using the CRISPR/Cas9 technology is its accuracy as well as its efficiency in editing the specific genomic sequences. It also shows very limited endogenous off site effects when compared to RNAi [11]. CRISPR is also comparatively cheaper to prepare and has a simple target design process.

CONCLUSION:

Cancer has been a challenge for many centuries to scientists , and despite all the treatments suggested, a holistic solution is not yet discovered for this complicated disease. THe recent studies on CRISPR look hopeful and there is a very good chance that a proper treatment for cancer using CRISPR/Cas9 as a molecular vector could be somewhere around the corner.

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