Crispr Cas System For Treatment Of Ovarian Cancer And Its Delivery Methods

Abstract

CRISPR/ Cas technology is a gene editing tool used in biotechnology and pharmaceuticals for studying diseases and its treatment. This paper majorly concentrates on ovarian cancer, its different types and the major genes involved in causing the disease. Few researches about how this CRISPR technology is used for treatment of ovarian cancer in-vitro has also been provided. What new treatment methodologies can be developed using CRISPR/Cas technology? Due to inefficient delivery system of this CRISPR, what research has already been performed to test delivery methods and followed by few examples about what new methods or delivery system can be used for this treatment method to show efficacy in clinical trials.

1. Introduction:

Advances in field of biotechnology and pharmacology has led to development of various approaches like CAR T cell therapy, chemotherapy, immunotherapy for treatment of cancer. Apart from these therapies, gene editing therapy has opened new gates for treatment. [1] Gene editing technology has been developed from last two decades starting from Zinc Finger Nucleases (ZFN's) [2] made up of zinc finger protein and nonspecific FOK1 DNA cleavage domain [1], TALEN's (Transcription activator like effector nucleases) and CRISPR technology which is widely used due to its high efficiency, cheap, high throughput and genetic and epigenetic editing. [2]

In cancer research, CRISPR technology has shown great promise in treatment by manipulating cancer genome, epigenome editing, inactivation of carcinogenic viral infections [3], knockout specific genes or repairing mutations [4] An intracellular delivery system is to be developed using CRISPR Cas plasmids as cancer derived exosomes for treatment of ovarian cancer. [5]

1.1 Type 2 CRISPR Cas9 :

Most widely studied and known CRISPR/Cas system is type 2. The type 2 CRISPR (Clustered Regularly Interspaced Palindromic sequence)/ Cas system is a revolutionized gene editing technology. It has two major components : sgRNA (guide RNA) and Cas 9 enzyme (nuclease). Guide RNA guides the Cas 9 enzyme to the site of genomic DNA where it must cut. The complex sgRNA/Cas 9 recognizes the target by the presence of 3' PAM (Protospacer Adjacent Motif) located on the genomic sequence. After its correctly recognized, the nuclease enzyme creates a double strand break (DSB's) which needs to be repaired via break repair mechanisms creating insertion or deletion (indels). [6]

This CRISPR technology can be used to modify tumor cells to undergo apoptosis, modification of antitumor therapy using monoclonal antibody and adoptive immunotherapy. [1] Using this technology in multiple ways can be effective treatment of cancers. In this paper, we will talk about how ovarian cancer can be treated using CRISPR technology and what innovations can

be made to effectively use in future for clinical trials.

1.2 Ovarian Cancer:

As per WHO, every year 2,25,500 cases of ovarian cancer are diagnosed, and 1,40,200 cases fail to survive. [7] Ovarian Cancer is the 5th leading cause of cancer among women. It is the most lethal and malignant form of cancer. Epithelial ovarian cancer (EOC) is the most common form of ovarian cancer based on histology which has survival rate of 45.6% which decreases to 30% as the stage of cancer gets advanced. [8] EOC are further divided into serous, endometrial and clear cell type. Serous type ovarian cancers are further classified into high grade and low-grade serous carcinoma. [7]

Mutations observed in ovarian cancer is mainly germline for BRCA 1 and BRCA2 genes. Other mutations in genes like RAD51, RAD50 are also possible. 14% of all cases in EOC are due to BRCA1 and BRCA2 mutation, out of which BRCA1 contributes a bit more. Low-grade serous carcinoma has mutations in KRAS and BRAF and are not associated with BRAC genes. High-grade serous ovarian cancer (HGSOC) also have mutations in BRCA1 genes.[9] BRCA 1 gene is a nuclear phosphoprotein acting as a tumor suppressor gene by maintaining genomic stability.[10] BRCA2 is involved in DNA repair mechanisms via homologous recombination pathway that resolves the double stranded breaks. [11] Risk of causing ovarian cancer is 40% if the women have BRCA1 mutation.[9] Drugs like Niraparib [15] and Olaparib have been developed for treatment of ovarian cancer having BRCA1 and BRCA2 mutations. [16] Niraparib has side effects of moderate bone marrow toxicity. [15] Olaparib has side effects like anemia.[16]

1.3 PD1/PD-L1:

PD1 (Programmed Death ligand 1) is a transmembrane protein expressed in normal tissues on T and B cells for immune checkpoint mechanisms. Its expression is found to be increased on the tumor cells and its binding to PD-L1 ligand leads to inhibition of T cells immune system response towards tumor growth. [12] Overexpression of PD1 and its ligand is also observed in ovarian tumors. [13] Monotherapy using PD1 blockade was performed which showed overall response rate of 10-15%, which was modest.[13] Thus, better therapy must be developed for treatment of cancer by targeting PD1.

Thus, using gene editing technology like CRISPR we can develop methods to treat ovarian cancer by targeting genes like BRCA1/BRCA2 and PD1 can be helpful.

2. Some CRISPR Cas technology that are undergoing research for treatment of ovarian cancer are as follows:

2.1 Lentiviral CRISPR Cas 9 approach to mutate miRNA 21 in ovarian cancer leading to inhibition of epithelial – mesenchymal transition (EMT) pathway

miRNA overexpression is one of biomarker of ovarian cancer. Thus, they developed CRISPR / Cas 9 system for editing miRNA 21 gene in pre-miRNA sequence of ovarian cancer cells (OVCAR3 and SKOV3). They used four different gRNA sequence to mutate the gene and each of them proved to create either a base pair deletion or/and insertion. This mutation lead to

inhibition of cell proliferation, migration and invasion in ovarian cancer cells and increased the efficacy of chemotherapeutic drug treatment. This was first study to be showing promising results and can be used for treatment of cancer. [17] They used lentiviral method to transfer them into cells, but this cannot completely be used for introducing in humans for clinical trials.

2.2 CRISPR/ Cas 9 system for in-vivo treatment of ovarian cancer:

Overexpression of DNMT1 gene was found to inactivate tumor suppressor gene leading to tumorigenesis. High levels of this gene are observed in patients who had poor prognosis of this type of cancer. Various studies have also shown that inhibition of DNMT1 suppressed the growth of ovarian cancer cells. Thus, they wanted to develop a targeted therapy by editing DNMT1 gene using CRISPR/Cas 9 system. They used SKOV 3 ovarian cancer cells , prepared four gRNA specific for gene and transfected via F-LP (Folate receptor– targeted cationic liposome. They used this system for delivery because it was non-viral and met requirements of Cas9 delivery system. This study was performed both in-vivo and in-vitro systems. In-vitro studies showed that CRISPR system produced 28.6% indels in endogenous DNMT1 gene using FL-P/gRNA (DNMT1) and the F-LP delivery system was efficient in-vitro. In-vivo studies in mice, FL-P/gDNMT1 was introduced intraperitoneally in tumor model mice ,tumor inhibition rate was found to be 84%. Low expression in DNMT 1 leads to decrease in genome methylation and necrosis of tumor cells. FL-P was found to be an efficient delivery system for treatment strategy. [18].

Very few studies have been performed using CRISPR technology for combating ovarian cancer. The above studies mainly involve using CRISPR knockout method to create mutations in gene which are overexpressed. Other ways in which CRISPR technology can be used are by knock-in genes or even short sequences or even combinational therapy of CRISPR and ABE's (Adenine Base editors). New ideas can be developed about how to use this CRISPR in other ways for treatment. With few other types of cancer studies being done using CRISPR technology, we can think of more ideas specific for treatment of ovarian cancer.

3. What can be future idea for using CRISPR/Cas as treatment regime:

3.1 Combinational therapy (In-vitro) : Knock in 'AG' nucleotides in BRCA1 gene and create mutations in PD1 using ABE's 7.10

As I mentioned above, important genes and a ligand which are major causes of ovarian cancer. I thought of developing a combinational therapy of CRISPR system. We can target both by using various approaches of CRISPR system.

BRCA1 gene has mutations like 185delAG (deletion of nucleotides AG at 185th position in gene) , 188 del11, and 5382insC (insertion of C nucleotide at 5382th position of BRCA1 gene) with ovarian cancer patients. This mutation was observed in 60% of ovarian cancer patients and among that 6.7% were for 185delAG. So, we are going to target this mutation using CRISPR/Cas knock-in strategy. We can develop a short sequence of gRNA can be called as og-RNA (oligo-guide RNA- short size) near to the 185th position of gene having a PAM sequence. Using benchling or some other CRISPR application, we can introduce this short mutation in the sequence and develop og-RNA sequence.

The next target is the PD-1 gene, we can create mutation in PD 1 gene of tumor cells so that it level of expression is reduced. This can be performed using CRISPR based ABE's (Adenine base editors).

ABE has hypothetical deoxyadenosine deaminase and a catalytically impaired cas9 and they target the sequence of interest using the gRNA sequence. This creates a small bubble of ssDNA and causes conversion of AT base pair to GC base pair. This technology is widely used for correcting mutations in diseases and suppress the gene by creating mutations in human cells. Many variants of ABE's are developed for various functions. ABE 7.10 is the most efficient and has broad range of sequence ability. [19]

We thought of using ABE variant 7.10 along with CRISPR Cas for creating mutations in PD1 gene. We can develop specific gRNA sequence towards the exon which is involved in inhibition of T cell proliferation and create mutation. We can hypothesize that this mutation is created at any AT nucleotides in the sequence and will have downstream effect of lower or no expression of this gene on tumor cells.

For BRCA1 created og-RNA, we introduce in Cas9 expressing plasmids and transfect it in the cancer cells. Along with that, we will also introduce variant of CRISPR- ABE 7.10 containing plasmid in the same ovarian cancer cells. This can be considered like co-infection of two plasmids to manipulate the gene in cancer cells to make them less aggressive if the knock-in and editing mutations have occurred in the cancer cells.

To check, if knock-in of AG has occurred using og-RNA we can perform Western Blot and to check if CRISPR ABE's 7.10 have worked we can perform an experiment wherein we develop a culture plate having the transfected cancer cells and T cells (introduced from outside) and check if the PD1 of T cells can bind to the PD-L1 ligand on tumor cells using ICC (immunocytochemistry).

Thus, introducing two different variants of CRISPR in one cell lines, we can create or delete mutations for the respective genes thus can help in combating this disease. [Figure3]

4. Delivery system for CRISPR/Cas :

There are 3 major methods which are used for delivery of CRISPR: Physical methods, viral delivery method and non-viral delivery method. Each of these methods have their own advantages and disadvantages based on whether it is used for in-vitro or in-vivo delivery of CRISPR/Cas. Microinjection is a physical method of delivery used for knockout of genes in cynomolgus monkey at zygote level and they were able to achieve the mutation, but it was observed to have detrimental effects on the embryos.[20] Electroporation is also method which is used as delivery system for CRISPR/Cas for developing model of Medulloblastoma and it was observed to be efficient in developing mouse. [21] One of the most widely used method is viral delivery system using AAV (Adeno Associated virus) due to low immunogenicity, high packaging capacity, non- integrating, and transient expression. It is already used for editing genome in mice in-vivo.[22] Non-viral vectors have been most easy and commercial system to be used for delivery of CRISPR/Cas system in-vitro like Cationic polymer PEI-beta cyclodextrin and gold nanoclusters. Recently, new delivery systems like polymeric nanoparticles containing PEG-PLGA and cationic lipid for cancer treatment, SKOV3 derived exosomes delivery for

treatment of ovarian cancer have been developed for in-vivo studies. Receptor mediated in-vivo delivery system like folate modified liposomes, non-cationic and deformable nanolipogel, Semiconducting polymer brush, Cationic-polymer coated nanorod are developed. [23]

Many different types of delivery system are undergoing research but none of them have reached clinical trials due to major limitations. Only, CAR-T cell therapy (CRISPR/Cas) is currently undergoing clinical trials phase 1 for solid tumors.[24] CRISPR/Cas system can also be manipulated in a way that it is not detrimental to health as one example I provided was using ABE's. More new methods and innovations can be made for efficient delivery of CRISPR / Cas. Thus, more research is needed to develop an efficient delivery system for CRISPR/ Cas for treatment of ovarian cancer and even cancers.