**Advances in Scientific Technology with Gene Editing Tools and its Relation to Cancer Treatment**

Author: Mackenzie Merritt
Major: Microbiology/ Cell & Molecular Biology
Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA

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**Recently, new advances in gene editing technology might make it possible for a new treatment of cancer to emerge. CRISPR, the gene editing technology in question, has been used in studies to see if it could be a new way to treat some types of cancer. The CRISPR-CAS9 system is a bacterial cell mechanism used to fight against viruses that are attacking the bacterial cells. The CRISPR-CAS9 system has been modified to where it can make modifications to human DNA. These modifications are made by CRISPR guiding the enzyme Cas9 to the gene that is to be modified, that is, the gene that is to be cut out and replaced. If scientists were able to utilize this gene-editing tool, many different diseases could be treated, and possibly eradicated. Research on how to use this gene-editing technology in humans has recently been carried out to see if cancer, and other diseases, can be treated using this technique. Currently, CRISPR technology has been successful in suppressing HIV cell replication, and has destroyed some antibiotic resistant bacteria. However, delivery strategies of the CRISPR-CAS9 system are still being researched and scientists also do not know the long-term effects of this type of gene editing.**

**Introduction**

There are many ways that the CRISPR gene editing technology is being used in research today. It is commonly being used in research to see what effects it can have on cancer. The CRISPR-Cas9 mechanism was discovered in the organism *Streptococcus pyogenes*. This organism and others use this mechanism to protect itself from viral infections within its own cells (Ormond, Kelly E et al). The CRISPR-Cas9 gene editing system is composed of a target-specific single guide RNA (sgRNA) and a Cas9 endonuclease. To utilize this gene editing system in their own research, researchers create a small piece of RNA with a short "guide" sequence that binds to a specific target sequence of DNA in a genome. The RNA binds to the Cas9 enzyme, which allows the Cas9 enzyme to recognize the targeted DNA sequence. This enables the enzyme to cleave the DNA on both strands at that targeted location. Then the cell’s normal DNA repair machinery repairs the break in the DNA (Ormond, Kelly E et al). The outcome from this repair process is often the introduction of a mutation, mostly the deletion of some DNA at the target site. If a separately engineered ‘‘donor’’ DNA fragment is also provided, the repair machinery can use this as a template to fix the DNA break—thus, the engineered DNA molecule can allow new sequences to be introduced at the target site (Ormond, Kelly E et al). There are two possible repair pathways that that can stimulate genome-editing: nonhomologous end-joining, resulting in insertions and deletions, or homology-directed repair, resulting in precise sequence substitution in the presence of a repair template (Platt, Randall J et al). These processes are what is used to edit the gene, and introduce the gene sequence that could potentially correct the mutations that are causing the disease. There are a wide variety of diseases that could utilize the CRISPR/Cas9 system to repair specific mutations that have to do with that certain disease. Besides being a possible cancer treatment or cure, CRISPR can be used to treat other diseases as well. These diseases include HIV, Huntington’s, muscular dystrophy, and even sickle cell anemia. It has also been utilized in human germline genome editing. In early 2015, the first study demonstrating that CRISPR/Cas9 could be used to modify genes in early-stage human embryos was published. Those embryos were not capable of developing to term, however, the work demonstrated that genome editing in embryos could be readily performed (Ormond, Kelly E et al). In another study, CRISPR/Cas9 was capable of inhibiting multiple steps of HIV-1 infection. This inhibition resulted not only from insertions and deletions that were introduced into viral DNA due to Cas9 cleavage, but also from the marked decrease in the levels of the late viral DNA products and the integrated viral DNA (Yin, Lijuan et al). Some promising results have been seen with these diseases, but what about cancer? There are many studies that have been conducted that test aspects of the CRISPR-Cas9 gene editing technology. Some studies have looked into ways to efficiently deliver the gene editing system into the cells. Others have focused on using the gene editing to modify certain features of cancer cells, so that their cell function is modified. This would alter the effectiveness of the growth of the cancer cell. Most of these studies have shown ways that this gene editing tool can be utilized to slow down the degrading effects of cancer cells, but not necessarily a way to totally eradicate the cancer cells. Even if the studies do not necessarily point to a cure, the studies that are using CRISPR are advancing our knowledge with how gene functions work in normal, and diseased conditions.

**Recent Progress**

Recently, there has been progress on developing techniques with CRISPR gene editing technology to treat cancer. It is well known that one of the major treatment hurdles of advanced-stage cancer is localized and distant tumor cell metastasis, resulting from vascular infiltration or penetration of anatomic boundaries (Ormond, Kelly E et al). In regards to tumor growth, one study used CRISPR in order to engineer therapy-sensitive cancer cells for self-targeting of primary and metastatic tumors by expressing cytotoxic molecules. There were three scenarios tested that feature the potential for self-targeting that included: Local treatment of surgically controllable (primary nodular) tumor recurrence, local treatment of recurrent (primarily invasive) cancers for which surgical debulking is not indicated, and systemic treatment for disseminated/metastatic disease (Clemens Reinshagen, et al). The CRISPR engineered cells were also able to avoid immune-mediated responses that would prematurely knock out the therapeutic genes. The CRISPR-modified therapeutic cancer cells were also found to directly kill self-cells via TRAIL-induced apoptosis in vitro and in vivo and that, in combination with their self-homing properties, these effects increase the survival of mice bearing autologous recurrent or metastatic tumor deposits (Clemens Reinshagen, et al). The TRAIL’s receptor-targeted properties have the advantage of not inducing cytotoxicity in normal cells. As compared to the cytokine that is typically engineered to secrete from tumor cells, TNF, which can cause inflammation and tissue degeneration (Clemens Reinshagen, et al ). The fact that antitumor efficacy was seen in vitro and in vivo, is a great accomplishment. This is because one of the difficulties of CRISPR-Cas9 has been with finding the right delivery method. Commonly used delivery systems based on lentiviral and adeno-associated viral (AAV) vectors have limited packaging capacity (Platt, Randall J et al), which renders it challenging for incorporation of Cas9 along with sgRNA expression cassettes and necessary genetic elements (i.e., promoters, fluorescent proteins, and polyadenylation sequences). Higher capacity viral vectors, such as adenovirus, can be used to deliver large transgenes, but generally have higher immunogenicity, limited cell type specificity, and tissue tropism (Platt, Randall J et al). Most recently, hydrodynamic injection, which can accommodate the large transgene size of Cas9, has been employed to achieve Cas9- mediated genome editing in the mouse liver in vivo, albeit with low editing efficiencies, and is primarily applicable to hepatocytes within the mouse liver (Platt, Randall J et al). Given these challenges, there is an urgent need for a more versatile system to enable efficient Cas9-mediated genome editing for in vivo applications (Platt, Randall J et al). This was why the modification of mice was necessary, so that a more efficient delivery method could be developed. In the study by Platt and other authors, they used Cre-dependent Cas9 knockin mice as their animal model to surpass delivery difficulties. Specifically, they used these mice to generate loss-of-function mutations in tumor suppressor genes as well as gain-of-function mutations in a proto-oncogene (Platt, Randall J et al). By using these mice, they were able to model the top three mutated genes seen in a lung adenocarcinoma: KRAS, p53, and LKB1 (Platt, Randall J et al). It is important to note that the prolonged effect of Cas9 expression was evaluated in this study, and no significant differences between the mice models and the wild type was noticed. For example, at the cellular level there were no morphological abnormalities or upregulation in DNA damage and apoptosis markers. There were no differences between wild-type and Cas9-expressing neurons. The result of the crossing of a Cre-dependent Cas9 mouse to a β-actin Cre driver, the resulted in progenies that were viable, and the Cas9 expression was seen in the progenies as well (Platt, Randall J et al). The success seen in this study can lead to the possible rapid detection of gene mutations that are seen in a wide variety of diseases, including cancer. The results seen in just these two studies show great promise to what the CRISPR-Cas9 gene editing technology has to offer.

**Discussion**

While the results seen in the two studies mentioned in the last section seem promising, there is still a great amount of research that needs to be completed. The delivery method of the Cas9 system has not been made completely safe or efficient. The delivery methods range from having limited packaging abilities, to having greater packaging abilities that come with problems of their own. However, researchers are continuing to research on how to alter this. As we saw in one of the studies that were mentioned. We also do not know the long term effects that this gene editing technology will have on our cells. Although CRISPR/Cas9 is a highly efficient mechanism for various cell types, it is infrequently 100% accurate at introducing the gene alterations at the target site, although double-digit percentages are often collected during research (Ormond, Kelly E et al). CRISPR has also been known to cut genes that are very similar to the gene that they are supposed to cut as well. These off-target effects could be a major issue at hand. If the incorrect gene is cleaved, it could lead to dangerous mutations within the cells. In terms of the possibility of this mechanism being used on humans for germline editing, there is still a great deal about the process that needs to be perfected. Without knowing all of the risks of which mutations could be introduced, knowing the effects of those, clinical settings using this mechanism will probably be put on hold. Despite all of the research that has been done, and the scientific advances that have been made, cancer remains the second most common cause of death in the western world. It accounts for nearly one of every four deaths in the United States (Ormond, Kelly E et al). The need for more therapeutic approaches are great, especially in cases of recurrent and metastatic disease, where standard therapy has failed (Ormond, Kelly E et al). Though there is still a great deal of research to be done on this gene editing technology, the positive results that we have gotten thus far in research are very promising. With more research, and trials, CRISPR gene editing could be the thing that can help treat cancer.

**References**

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