CRISPR Therapeutics for Pancreatic Cancer

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Abstract

Pancreatic cancer is one of the most fatal cancers with a five-year survival rate of just 7.2%. The current treatments available for pancreatic cancer are often unsuccessful, creating a pressing need for more effective therapeutic options. CRISPR gene editing has emerged as a powerful tool with the potential to revolutionize cancer treatment strategies. Experiments using CRISPR in animal models have proven successful in stopping pancreatic cancer progression. This versatile technology can be used to edit, silence, or disrupt genes, presenting a novel avenue for treating genetic cases of pancreatic cancer in patients. In this review, I discuss the history behind CRISPR and its promising role in reshaping therapeutic approaches for pancreatic cancer.

Introduction

Pancreatic cancer, also known as ductal carcinoma, is a tumor that starts in the pancreas (usually the pancreatic duct) and can grow to affect other parts of the body, such as pushing into the liver and causing liver failure (Colombia Surgery). The pancreas is made up of exocrine cells, and a pancreatic tumor forms when those exocrine cells grow at an uncontrollable rate (American Cancer Society, 2024). The most common type of pancreatic cancer is known as Adenocarcinoma, which accounts for 95% of all pancreatic cancers (American Cancer Society, 2024).

Annually, 64,000 people are diagnosed with pancreatic cancer, and 79% of cases are fatal (American Cancer Society, 2024). Although pancreatic cancer is only 3% of diagnosed cancers yearly, it accounts for 7% of all cancer deaths (American Cancer Society, 2024). For metastatic pancreatic tumors, the survival rate is merely 2.4% (Colombia Surgery). The most common symptoms of pancreatic cancer include jaundice, loss of appetite, fatigue, weight loss, back and abdomen pain, liver and gallbladder enlargement, and blood clots (American Cancer Society, 2024; PDQ Adult Treatment Editorial Board, 2024). Unfortunately, these symptoms are not specific to pancreatic cancer, and by the time they are detected, the cancer has already started to spread. For these reasons, pancreatic cancer is extremely difficult to diagnose early, which is one of the main contributing factors to the low survival rate.

Although the main cause of pancreatic cancer is not fully understood, there are a variety of factors that can contribute to tumor development. Some of these factors include environment, personal health choices, and genetic mutations (Colombia Surgery). Inherited genetic mutations cause 15% of all pancreatic cancers. For example, the BRCA1 and BRCA2 genes, while known to heighten the risk of ovarian and breast cancer, can also heighten an individual's risk for pancreatic cancer (Colombia Surgery). Also, exposure to cancer-causing chemicals, such as tobacco smoke, can result in acquired mutations, which puts people at a significantly higher risk for developing pancreatic cancer (American Cancer Society, 2024).

While a cure for all pancreatic cancer is not yet medically obtainable, one promising treatment option for cancers caused by genetic mutations (both inherited and acquired) is gene therapy. The main goal of gene therapy is to replace defective genes with healthy genes, with the intention of preventing a tumor from continuing to divide or preventing an individual from developing cancer altogether (Das et al., 2015).

A novel method that can be used for gene therapy is CRISPR. CRISPR (clusters of regularly interspaced short palindromic repeats) is a gene editing tool that uses a Cas9 protein and a guide RNA to target specific genes of interest. The guide RNA is specifically designed to be complementary to the gene of interest so that the Cas9 protein can create a double-stranded break. Once the gene is "cut" via the double-stranded break, a donor DNA can be used to correct any mutations within that gene. This mechanism, first identified in bacteria as a form of immunity against viruses (Yang et al., 2019), has been manipulated for use in the laboratory.

Scientists can now use CRISPR on human cells - proposing a promising outlook for the future of cancer treatments. Many scientists hope that this specific gene therapy treatment will be the first approach to treating all different types of cancers (and even preventing them) (Das et al., 2015). In this review, I will discuss how CRISPR can be integrated with gene therapy as a potential treatment option for pancreatic cancer.

What is CRISPR?

CRISPR (clustered regularly interspaced short palindromic repeats) is an adaptive immune system that bacteria and archaea use to fight off bacteriophages and other viruses that infect them. A palindromic sequence is a sequence that is the same backward as it is forward - in CRISPR's case, it means that both a DNA strand and its complementary strand are the same when read in the 5' - 3' directions. CRISPR was originally discovered in 1987 in *E. coli* (Javed et al., 2018; Lino et al., 2018). It was noted that bacteria and archaea have spacer sequences in their DNA that space out these palindromic sequences and that these spacers appeared after the organism was infected with a bacteriophage (Javed et al., 2018). 40% of bacteria and 90% of archaea were found to contain these spacer sequences; however, the reasoning behind this observation was still unknown at the time (Javed et al., 2018).

In 2002, scientists discovered a set of genes next to these CRISPR repeats, which were called CRISPR-associated systems or Cas (Lino et al., 2018). These genes were determined to be involved in regulating DNA replication and repair, as well as gene expression. A few years later, in 2005, it was uncovered that the spacer sequences within the bacterial CRISPR arrays are pieces of DNA from bacteriophages that previously infected the organism. It was concluded that the CRISPR system must be an adaptive immune system that is used to fight bacteriophages (Javed et al., 2018). When a bacteriophage infects a prokaryotic organism, the bacteria or archaea takes a small piece of DNA from that bacteriophage and incorporates it into the CRISPR locus, which creates a CRISPR array. The array then goes through transcription to create a CRISPR RNA (crRNA) strand.

The Cas genes encode for a large protein with endonuclease activity (ability to cut DNA). The Cas nuclease scans for invading DNA and disables it by creating double-stranded breaks (Lino et al., 2018). The method by which the Cas protein can do this is by recognizing the crRNA and its adjacent spacer sequence called PAM. Together with a trans-CRISPR RNA (TracrRNA) strand, the Cas protein forms an effector complex with the CRISPR RNAs. If viral DNA is found to be complementary to the RNA spacer sequence within the effector complex, the Cas nuclease will create these double-stranded breaks. Now, the virus is neutralized, as the bacteria can no longer transcribe the virus' DNA to create more viral particles.

A major breakthrough occurred in 2012 when scientists Jennifer Doudna and Emmanuelle Charpentier discovered that crRNA and tracrRNA could be fused to form a single guide RNA (sgRNA) (Broad Institute). They uncovered the full mechanism of CRISPR, earning

them a Nobel Prize in Chemistry, and then harnessed its abilities for use in eukaryotic cells (Javed et al., 2018). From there, CRISPR began to revolutionize the field of gene editing.

Due to recent advancements in CRISPR technology, CRISPR has started to be used to treat genetic diseases that have known mutations. This is because scientists can create a sgRNA in a lab, and when complexed with a Cas9 protein, it can cleave the DNA just like it would in bacteria. Therefore, if the sgRNA is synthesized with the correct complementary sequence, it can be inserted into a cell with Cas9 in order to direct Cas9 to the appropriate gene of interest. This technique can be used to target Cas9 to a specific genetic mutation known to cause a particular genetic disease. This makes CRISPR an extremely crucial discovery for the gene therapy field.

One example of this advancement in technology is how CRISPR was successfully used in Huntington's disease. Huntington's disease has been observed for many years, and until CRISPR, scientists had yet to find a treatment. However, Huntington's disease has a known genetic base (a sequence of repeated CAG bases). Using this knowledge, an experiment was conducted that permanently inactivated the CAG base repeat from generating in a specific organism (Shin et al., 2016).

CRISPR has also been tested in its treatment ability for type 1 diabetes, amyotrophic lateral sclerosis, murine muscular dystrophy, acute kidney injury, and more (Knott & Doudna, 2018), all of which show hopeful outcomes for the future of their treatments. In addition, recent Cas9 development advancements are beginning to provide ways to further prevent DNA damage while using CRISPR in eukaryotic cells (Knott & Doudna, 2018). For the context of this paper, it is most important to address that another advancement in CRISPR technology was the development of T cells to engineer advanced immune systems that can help fight different cancers. Furthermore, CRISPR also provides the technology to possibly prevent an individual's risk of obtaining a certain cancer at all, depending on their risk for the cancer due to their genetic makeup (hereditary risk or chemically mutated risk) (Knott & Doudna, 2018).

CRISPR's Possible Use for Pancreatic Cancer Treatment

Pancreatic cancer is one of many cancers where CRISPR shows potential for successful treatment methods. Current treatments for pancreatic cancer are rarely ever permanent cures, making it one of the most fatal cancers to exist (Guo et al., 2013). Since it is mostly asymptomatic, by the time it is diagnosed, surgery is rarely an option due to it spreading to different organs throughout the body, and the recurrence rate is typically very high (Guo et al., 2013). Additionally, treatment using chemotherapy faces significant challenges when performed on late-stage pancreatic cancer because the pancreas is very resistant to chemotherapeutic drugs. The dosage needed to eradicate the pancreatic tumor would have a drastic effect on the patient's immune system, making it a very dangerous therapeutic option for those with pancreatic cancer. Due to these risks, chemotherapy is typically not an available option for patients (Guo et al., 2013). Radiation therapy has similar limitations, as the pancreas has very low levels of radiosensitivity, making the dosage needed for radiation very high. However, the organs surrounding the pancreas have a very high level of radiosensitivity, meaning that if radiation was performed on the pancreas, it is very likely the surrounding organs would suffer harsh side effects and damage (Guo et al., 2013). Immunotherapy is another option for pancreatic cancer, which depends on tumor antigens in order to activate the cell-mediated pathway of the immune system. This method can also consist of modified white blood cells that

are activated to fight the cancer cells. However, research currently lacks a pancreatic tumor-specific-antigen, which oftentimes makes immunotherapy unsuccessful (Guo et al., 2013).

Due to its ability to edit and correct known genetic mutations, CRISPR has the potential to create an entirely new method to help prevent and fight pancreatic cancer. A recent study focused on CRISPR's use against the KrasG12D mutation, which is a mutation found in 90% of PDAC tumors (Jiang et al., 2020). It was found that CRISPR was able to silence the expression of the mutation in the gene, which eliminated the possibility of mutated Kras proteins being created. Overall, this resulted in a significantly lower risk for pancreatic cancer. This was done using CasRX proteins, which are similar to the Cas9 proteins but bind to RNA instead of DNA. The CasRX protein binds to the specified gene (in this case, the KrasG12D gene), and degrades the mutated gene, which prevents the mutated protein from being produced (Jiang et al., 2020).

Another method that was found to be successful during a study of CRISPR in pancreatic cancer was the targeting of hypoxia-inducible factor-1α (HIF-1α). Using CRISPR, the levels of HIF-1α within the tumor were decreased, meaning that not only was the growth of the cancer decreased but so was the possibility of it spreading to other organs. This was done using lipid-based delivery methods that specifically targeted the cancer cells within the tumor (Li et al., 2019).

Another study used CRISPR to fight against tumors that are typically hard to fight because of their solid center. This solid center has an acidic, hypoxic, and necrotic environment. This environment attracts the bacteria Clostridium Novyi, which can be used to eliminate the tumor and also to strengthen the individual's immune system. Scientists therefore decided to use a modified version of Clostridum Novyi (called Clostridum Novyi-NT). While there is a major advantage of this modified bacteria because this version does not kill other cells, the disadvantage is that once it is injected into the bloodstream, it cannot stay in the tumor long enough to eliminate it, rendering the method useless. However, using CRISPR, scientists were able to add a gene to the bacterial DNA so that it would have the ability to attach itself to the tumor for longer periods of time. This method now has the potential to destroy a tumor successfully (Dailey et al., 2023). Pancreatic cancer is most times a solid tumor, meaning that this method is a possible new solution to fight against pancreatic tumors.

Ethical Concerns

CRISPR's revolutionary impact on the field of gene editing and therapy has brought significant attention to the ethical dilemmas surrounding genetic editing. The complexities and controversies regarding the ethics of CRISPR prompted the imposition of temporary and partial bans on the gene-editing tool in 2015 (Guttinger, 2018). These bans remain in effect until there is a consensus on the circumstances under which gene editing is deemed beneficial and where it crosses ethical boundaries. This issue is debated by many major organizations, including the World Health Organization (WHO) and the United Nations Educational, Scientific and Cultural Organization (UNESCO) (Cribbs et al., 2017). To date, 19 countries have completely banned germline cell editing and clinical trials involving CRISPR. Some countries, such as the U.S. and China, permit CRISPR for non-reproductive purposes only (Guttinger, 2018). The only legalized form of gene editing is the Mitochondrial Replacement Technique (MRT), which is used solely to prevent mitochondrial-based diseases from being passed on from mother to child (Claiborne et al., 2016).

The controversy surrounding CRISPR gene editing primarily involves the use of germline cells and embryo editing. An infamous example of this is the birth of twins, which were the result of Dr. He editing human embryos to confer immunity to HIV. Dr. He targeted the *CCR5* gene, which encodes for a protein that HIV strands use to enter cells (Brown and Rose, 2019). He hypothesized that if he could inhibit this gene from creating the CCR5 protein, then HIV would be unable to enter the cell. This would therefore make individuals immune to most versions of HIV. Dr. He's experiment raised a myriad of ethical questions and ultimately led to his imprisonment. He not only violated the imposed ban on CRISPR in human embryos but there was also significant concern regarding his choice to target HIV. There was a high likelihood that the children would not have developed HIV naturally. To test whether he successfully edited the embryos and conferred immunity to HIV, Dr. He would have to intentionally expose the children to HIV. This poses an extremely unethical practice.

An important risk factor regarding CRISPR that is also debated is the limitations of the tool due to specificity issues. The main concern is that the single guide RNA of the CRISPR tool does not need an exact match for Cas9 to bind and create a double-stranded break (Guttinger, 2018). The human body contains around 30,000 genes, which are made up of the same four nucleotides. This means there is a high potential for repetitive or closely related gene sequences, leading to the possibility of CRISPR editing the wrong gene. This not only fails to cure the patient but it also poses a high risk of causing other genetic diseases, including various types of cancer. Moreover, the limited understanding of human genes raises the possibility that editing a disease-causing gene may reveal its involvement in other essential bodily processes, potentially causing unintended problems. Furthermore, not all genes are expressed, which leaves the possibility that a CRISPR side effect might not become apparent until the next generation, affecting many people before the problems are recognized.

In conclusion, the transformative potential of CRISPR technology in gene editing and therapy has sparked complex ethical debates, leading to global regulatory measures. As the scientific community grapples with the challenges of specificity, unintended consequences, and the responsibility associated with altering the human germline, ongoing discussions and advancements aim to strike a delicate balance between the promise of medical breakthroughs and the ethical considerations that surround the revolutionary CRISPR technology.

Conclusion

As CRISPR revolutionizes the field of gene editing, it has proven to be a potential treatment for many different genetic diseases. Given that pancreatic cancer has a 5-year survival rate of just 5%, and it is incredibly hard to treat because it is often caught too late, the CRISPR/Cas9 technology has the potential to act as an additional therapeutic in order to raise the survival rate and help patients who might not have originally had a chance at recovery. However, it is important to note that although there are many advantages to CRISPR, it is essential that the levels at which CRISPR is practiced and applied are limited due to current rules regarding the ethics of CRISPR. These rules stem from CRISPR's limitations, such as its low specificity. These off-target effects make it dangerous to use currently; however, the presence of CRISPR in modern-day therapeutics is relatively new and therefore has an extraordinary potential to be used in the future as a main therapeutic for diseases.

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