CRISPR-Based Therapy to Reduce MUC16 Expression in PDAC Patients

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) has one of the lowest survival rates among all cancer types, with an overall five-year relative survival rate of less than 5%. One of the causes stems from overexpression of the MUC16 gene, observed in about 20% of PDAC tumors. MUC16 is a large glycoprotein that forms a protective barrier around various epithelial cells and regulates processes such as cell proliferation and apoptosis resistance. When overexpressed, it enables cancer cells to evade immune detection, promoting metastasis. Some of the current treatment methods for PDAC include combinations of surgery, chemotherapy, radiation, targeted therapy, and immunotherapy. However, MUC16 overexpression causes resistance to chemotherapy and other treatment options, highlighting a need for alternative therapeutic strategies. Among various emerging approaches, CRISPR has gained attention as a novel gene editing tool that enables the precise modifications of DNA and holds potential to drastically improve cancer treatment. In this review, we discuss the use of CRISPR as a treatment strategy to reduce the expression of MUC16 in PDAC patients, exploring its potential to overcome therapy resistance and suppress metastasis.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a type of pancreatic cancer that forms in the exocrine cells of the pancreatic ducts. PDAC accounts for more than 90% of all pancreatic tumors (Kleeff et al., 2016). Despite advances in modern oncology, PDAC continues to have one of the lowest survival rates among all cancers with a five-year relative survival rate of less than 5% (Bengtsson et al., 2020). This is largely because it is often diagnosed at advanced stages, frequently at stage 4. At this point, the cancer has usually metastisized, leading to a devastating prognosis with survival typically ranging from four to six months after diagnosis (Chari et al., 2015). PDAC also has one of the highest recurrence rates of all cancers due to its high rate of perineural and venous invasion, allowing the cancer to travel through the vast nerve and vascular network. This further contributes to its position as the third leading cause of cancer-related deaths in the US (Siegel et al., 2020).

Among the genetic drivers of this disease, mutations in the MUC16 gene were found in about 20% of PDAC tumor samples (King et al., 2017). MUC16, a type 1 transmembrane mucin, is a large glycoprotein that forms a protective barrier around various epithelial cells and regulates processes such as cell proliferation, apoptosis resistance, and drug entry into the cell. MUC16 has been shown to be overexpressed in numerous cancer types. Its overexpression promotes tumor growth, enhances metastasis, and increases drug resistance (Zhang et al., 2024). Due to its elevated expression in various cancers, including PDAC, it has the potential to be a key biomarker for cancer diagnosis and a target for cancer therapies.

Current treatment options for PDAC include surgery, radiation, chemotherapy, targeted therapy, and immunotherapy, but survival rates remain low. This could be due, in part, to MUC16 overexpression limiting treatment success and long-term survival. Specifically, overexpression of MUC16 promotes chemotherapy resistance by suppressing the p53 gene and activating the JAK2/STAT3 signaling pathway, which is known to promote cell growth and suppress apoptosis in cancer cells (Lakshmanan et al., 2017). MUC16 also impairs the ability of chemotherapy drugs to interact with the plasma membrane of cancer cells. Additionally, this shield that MUC16 is creating around the cancer cells protects the cells from immune attacks, increasing their proliferation and limiting treatment options (Liu et al., 2016). This highlights the urgent need for new therapeutic strategies that can directly target the molecular components that are driving the progression of PDAC.

One promising approach is the application of CRISPR, a gene-editing technology that has continuously shown potential in experimental cancer therapies. CRISPR has been used in recent studies to knock out oncogenes, increase tumor sensitivity to drugs, and enhance the immune system's ability to recognize cancer cells. These applications demonstrate the ability of CRISPR to target specific genetic promoters of tumor growth and drug resistance.

This review will discuss the potential of CRISPR-based therapies as a novel treatment strategy for targeting MUC16 mutations in PDAC patients.

Main Text

Introduction to CRISPR

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a gene editing tool that has the ability to cut a target DNA sequence based on a customizable guide. CRISPR was originally discovered as an adaptive immune system used by bacteria (Synthego, n.d.). Scientists have since engineered CRISPR for use in mammalian cells, allowing for precise targeting of genes. CRISPR consists of two parts, a guide RNA sequence (gRNA) and a CRISPR-associated (Cas9) nuclease, an enzyme that can bind and create a double-stranded break in DNA. The gRNA directs the Cas nuclease to its target, and the Cas nuclease binds and cuts the DNA.

These two components work together to target a gene of interest through the use of the protospacer adjacent motif (PAM), a short sequence downstream of the target site. The gRNA is designed to be directly adjacent to a PAM site. When the gRNA-Cas9 complex binds to the region of interest, the Cas9 protein then scans the DNA for a PAM sequence (Synthego, n.d.). Once PAM is recognized, Cas9 checks the region upstream. If the target provided by the gRNA is located, then the Cas9 nuclease will create a double-stranded break in the DNA.

Cells have natural DNA repair pathways to fix broken DNA. The two that are most crucial to gene editing are non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is the most prevalent repair mechanism, which repairs DNA quickly but tends to lead to

the insertion or deletion of nucleotides, called INDELs (Pannunzio et al., 2017). This has the potential of resulting in a gene knockout or the inactivation of a gene. When a double-stranded break occurs, the Ku protein binds to the broken ends of the DNA. Afterwards, Ku recruits other proteins to bridge the gap between the ends to prevent the two strands from drifting apart. Various enzymes are then used to get rid of any protruding or incompatible DNA ends and to fill in any unwanted gaps, oftentimes introducing INDELs, while prepping the strands for ligation. During the ligation phase, the DNA ligase IV complex seals the processed ends together, completing the process of repair. Since NHEJ is the predominant method to repair double-stranded breaks in mammalian cells and is error-prone, scientists capitalize on this in order to knock out genes of interest.

The other predominant repair mechanism, homology-directed repair, utilizes homologous DNA templates to accurately fix double-stranded breaks in the genome (Haider & Mussolino, 2025). Once a double-stranded break is created in the cell's DNA, various enzymes shorten the broken DNA strands, creating single-stranded overhangs. The RAD51 protein binds to these overhangs and scans DNA to locate a suitable donor template. Once this template is found, RAD51-ssDNA filaments begin strand invasion, forming a displacement loop (D-loop) in the donor DNA. From here, one of two repair pathways occur. One of the pathways involves a DNA polymerase that utilizes the homologous template to synthesize new DNA by extending the invaded strand and repairing the break.The other pathway is called double-stranded break repair (DSBR). During DSBR, two Holliday junctions–four stranded DNA intermediates used during homologous recombination–are formed and later resolved to produce crossover or non-crossover products. Both of these HDR repair pathways allow for precise editing of the genome based on the donor template. To control what edit is made, researchers synthesize the donor template and provide it to the cells, along with the guide RNA and Cas9 protein. Thus, by harnessing HDR's ability to accurately repair DNA, scientists can use CRISPR/Cas9 to achieve precise and intentional modifications to the genome.

Use of CRISPR in MUC16

Due to CRISPR's precise gene editing ability, it is being used to aid in cancer treatment, including pancreatic cancers (Noormohamadi et al., 2025). Preclinical studies are exploring whether CRISPR can be used to suppress MUC16 overexpression as a therapeutic target for PDAC.

Preclinical Studies in PDAC Cell Lines

Multiple studies are exploring the use of CRISPR to knockout MUC16 in PDAC cancer cell lines. One study utilized shRNAs, short hairpin RNAs that silence gene expression, and CRISPR/Cas9 to knock down and knock out MUC16, respectively, in a capan-1 and colo-357

PDAC cell line (Muniyan et al., 2016). The reduced expression of MUC16 was confirmed by immunoblot and immunofluorescence analyses. Researchers observed a significant reduction in cell proliferation upon MUC16 knockdown compared to control PDAC cells. Specifically, in the colo-357 cells, MUC16 knockdown resulted in a reduction in the total cell count starting from day 1, and the cell growth rate was significantly reduced from day 4 until day 7. To see if this observation remained true in vivo, researchers xenografted the MUC16 knockdown cells into a mouse model of PDAC and compared the tumor to control xenografted PDAC cells. The results showed that MUC16 knockdown resulted in decreased tumor formation and metastasis compared to controls. Researchers next tested the effect of a full MUC16 knockdown in the PDAC cell line, which was achieved using CRISPR/Cas9. They analyzed the levels of tumor-associated carbohydrate antigens, which are structures on the surface of cancer cells that play roles in tumor growth, cell adhesion, and metastasis. Upon MUC16 knockout, the levels of tumor-associated carbohydrate antigens, T and Tn, significantly decreased in the PDAC cells. Overall, these results indicate that reducing or knocking out the expression of MUC16 can significantly reduce tumor formation, making it a viable target for cancer therapeutics.

A related study investigated the function of MUC16, by reducing or eliminating its expression in PDAC cell lines (Kaur et al., 2022). Researchers used shRNAs in SW1990 cells and CRISPR/Cas9 gene editing in CD18/HPAF cells. These methods allowed them to directly test how these pancreatic adenocarcinoma cancer cells behave when MUC16 is suppressed. When MUC16 was knocked down, the cells displayed more epithelial characteristics, such as higher expression of epithelial markers, which are linked together to stabilize organized tissue structure. The cells were also seen to display fewer mesenchymal traits, such as lower expression of markers associated with cell motility and invasiveness. This is important because the shift from epithelial to mesenchymal traits is a key step in cancer metastasis. Therefore, reducing MUC16 appears to reverse this, making cancer cells less invasive.

Loss of MUC16 also led to reduced activation of the FAK-Akt and ERK/MAPK pathways, which are crucial signaling pathways that drive cell survival, proliferation, and metastasis (Kaur et al., 2022). This shows that MUC16 helps fuel PDAC progression through these pathways.

The study further analyzed human tumor samples and found that PDAC liver metastases expressed much higher levels of MUC16 compared to primary pancreatic tumors, whereas lung metastases showed no significant difference from the primary site (Kaur et al., 2022). This highlights that MUC16 may play a particularly important role in promoting liver metastasis in PDAC, which is clinically relevant since the liver is a common metastatic site for this cancer. Additionally, patient-derived PDAC tumor organoids, 3D lab-grown tumor models, and normal pancreatic organoids were formed and stained for MUC16 and MUC16-Cter (carboxy-terminal domain of MUC16). The stains showed that MUC16 was strongly overexpressed in PDAC tumors but not in normal tissue. This reinforces MUC16 as a potential biomarker for distinguishing cancerous from noncancerous pancreatic tissue and suggests it could serve as a therapeutic target.

PDAC Mouse Models

In addition to in vitro testing, multiple studies are investigating the effects of MUC16 knockdown or knockout in in vivo PDAC models. One study found that deletion of MUC16 in a pancreatic cancer mouse model with mutations in KRAS and TRP53–tumor suppressor genes that drive tumor growth, metastasis, and chemoresistance when mutated, particularly in pancreatic cancers–led to a substantial decrease in tumor progression and prolonged overall survival rate in the mice (Kaur et al., 2022).

The researchers examined the Muc16 knockout pancreatic tumor tissue, which also harbors the TP53 and KRAS mutations, and found a decrease in tumor microenvironment factors and a significant reduction in liver and lung metastasis compared to the control pancreatic tumor tissue (Kaur et al., 2022). Next, organoids were created from the MUC16 knockout pancreatic tumor. These organoids displayed a significant decrease in growth rate compared to the control tumor organoids, indicating that loss of MUC16 significantly impairs tumor proliferation. By utilizing RNA-sequencing on MUC16 knockout pancreatic tumor tissues, it was seen that the cytoskeletal proteins Actg2, Myh11, and Pdlim3 were significantly downregulated in these MUC16 knockout tumors compared to control tumor tissue. Supporting this, analysis of the TCGA dataset demonstrated that ACTG2, MYH11, and PDLIM3 were substantially upregulated in PDAC patient samples compared to healthy individuals, suggesting that MUC16 positively regulates these genes during pancreatic tumor progression. Furthermore, at both the mRNA and protein levels showed that Actg2, Myh11, and Pdlim2 expression was significantly reduced in MUC16 knockout pancreatic tumors compared to control tumors. Similarly, ACTG2 and PDLIM3 protein expression were analyzed in human Capan1 CRISPR/Cas9 MUC16 knockout and SW1990 MUC16 knockdown pancreatic cancer cell lines relative to control cells. The downregulation of these cytoskeleton-associated proteins correlated with reduced metastatic potential, reinforcing the functional link between MUC16 and cancer cell motility. Overall, these findings suggest that MUC16 regulates pancreatic cancer progression by regulating cytoskeletal rearrangement through Actg2, Myh11, and Pdlim2. This modulation influences pancreatic cancer cell motility,endothelial cell migration, and tumor microenvironment dynamics, all of which are critical to processes in PDAC growth and metastasis. Importantly, these results strongly indicate that MUC16-driven regulation of cytoskeleton-associated genes is an essential mechanism in PDAC development and metastatic progression.

Furthermore, the progression of PDAC was studied using mice with disruption of C1galt1, an enzyme that synthesizes the “core 1” O-glycan structure (Pothuraju et al., 2018). In this study, mice with mutations in the C1galt1 gene were crossed with mice that had mutations in the TRP53, KRAS, and PDX1 genes (KPC mice). The growth and progression of pancreatic tumors in these mice were investigated and comparisons were made between the control KPC tumors and the tumors with mutations in all four genes (KPCC mice). Once collected, these pancreatic tumor tissues were analyzed through immunohistochemistry, immunofluorescence, and lectin staining. It was observed that KPCC mice had substantially shorter survival times, by about 102

days, compared to the control tumor mice who lived for about 200 days. It was also observed that the KPCC mice developed early pancreatic intraepithelial neoplasia at 3 weeks, PDAC at 5 weeks, and metastases at 10 weeks compared to control tumor mice. Additionally, tumors that developed in KPCC mice were seen to be more aggressive and invasive compared to the control. Researchers next used CRISPR/Cas9 gene editing system to disrupt C1GALT1 in human PDAC cells, and the levels of O-glycans were analyzed through lectin blotting, mass spectrometry, and lectin-pull down assay. The human PDAC cells with knockout of C1GALT1 had aberrant glycosylation of MUC16 and increased expression of genes that regulate tumorigenesis and metastasis, compared with the control cells. This strongly indicates that the loss of C1galt1 not only promotes the development of aggressive PDAC, but also increases metastasis. Knocking this gene out leads to increased tumorigenicity and reduction of O-glycosylation on MUC16, which has the potential to increase aggressiveness of PDAC. This study supports MUC16 and C1GALT1 as strong therapeutic targets for PDAC.

CAR T-Cell Therapy

Building on CRISPR-based approaches to reduce MUC16 expression, another promising strategy under investigation is the use of CAR T-cell therapy for PDAC. Chimeric Antigen Receptor T-cells (CAR T-cells), are genetically engineered white blood cells designed to specifically recognize and kill cancer cells. These engineered cells can generate potent anti-tumor immune responses, but their efficacy depends on the availability of a reliable tumor-associated antigen (TAA) to target. In a recent study, the retained ectodomain of Muc16, Muc16CD, is used as a TAA to guide CAR T-cell therapy in pancreatic cancer (PDAC) (Lin et al., 2024). The study demonstrated that Muc16CD-directed CAR T-cells could specifically recognize patient-derived pancreatic tumor cells and become activated in a polyfunctional matter, producing multiple effector molecules that enhance anti-tumor activity. In vivo experiments using a pancreatic tumor mouse model showed that these CAR T-cells induced significant tumor regression, improved tumor control, and prolonged overall survival compared with untreated controls. The therapy was effective even within the immunosuppressive pancreatic tumor microenvironment, which is typically a major barrier to treatment. These results indicate that targeting Muc16CD not only enables CAR T-cells to selectively kill PDAC cells but also sustains their functional activity in challenging tumor conditions, highlighting Muc16CD as a promising and clinically relevant target for CAR T-cell therapy in pancreatic cancer.

While CAR T-cell therapy can offer a new form of treatment for previously untreatable or difficult-to-treat disease, there are several limitations related to its durability, specificity, and off-target effects. CARs recognize specific antigens present on cancer cells, but some healthy cells may also express these antigens at low levels, which can lead to a lack of specificity and off-target effects (Korman et al., 2019). This reduces the therapy's overall safety while increasing the chances of adverse side effects. Additionally, CAR T-cells lose persistence over time, which can result in loss of effectiveness of the treatment or relapse for patients. These

challenges can result in less efficiency of CAR T-cell therapy; however, this can be overcome using CRISPR technologies.

CRISPR is being used to engineer CAR T-cells to have improved antigen recognition, allowing for increased specificity to cancer cells while reducing off-target effects (Dimitri et al., 2022). CRISPR can also be used to strengthen the CAR T-cells by having them co-express molecules that block inhibitory signals, secrete pro-inflammatory cytokines, or provide pro-survival signals. This allows for the CAR T-cells to withstand immunosuppressive signals in the tumor microenvironment, enhancing their overall tumor-killing abilities. Additionally, CRISPR allows for the modification of genes that cause T-cell exhaustion. By modulating these genes, CAR T-cells can have improved longevity in the body, increasing their overall persistence and thus enhancing the effectiveness of the treatment.

The combination of CRISPR technologies with CAR T-cell therapy represents a promising strategy for the treatment of PDAC. Enhancing CAR T-cells with CRISPR while targeting MUC16CD to direct them toward pancreatic tumor cells may significantly improve therapeutic outcomes and transform the current approach to PDAC care.

Human Clinical Trials

While the use of CAR T-cell therapy to target MUC16 has yet to be explored specifically in PDAC patients, there is an ongoing clinical trial targeting MUC16 for other cancers. This trial uses an experimental CAR T therapy called 27T51 as a potential treatment for adult females with recurrent or treatment-resistant epithelial ovarian, primary peritoneal, or fallopian tube cancers (U.S. National Library of Medicine, 2024). The study consists of two main parts: Phase 1a, which focuses on dose escalation, and Phase 1b, which focuses on dose expansion and combination therapy testing. The purpose of Phase 1a is to determine the safety and feasibility of administering 27T51 in humans, establish a maximum tolerated dose, and identify any dose-limiting toxicities. This phase also evaluates whether 27T51 can be reliably manufactured from patients' own T cells and safely infused back into them. Once an optimal dose is established, Phase 1b investigates how 27T51 performs both as a monotherapy and in combination with other cancer treatments, including cemiplimab, a PD-1 immune checkpoint inhibitor, and bevacizumab, an anti-VEGF antibody that targets tumor vasculature. These combination therapies aim to enhance CAR T-cells persistence and counteract the immunosuppressive tumor microenvironment, a major challenge shared by both ovarian and pancreatic cancers.

Eligible participants must have good performance status and meet organ function requirements to safely undergo leukapheresis, the process used to collect immune cells for CAR T-cell therapy manufacturing. Before receiving the infusion, patients typically undergo a lymphodepleting chemotherapy process to prepare their immune system for the CAR T-cells (Huang et al., 2024). Because CAR T-cell therapy can cause severe immune reactions, participants are closely monitored for cytokine release syndrome (CRS), neurotoxicity, and other

immune-related side effects. The study will further collect data on pharmacokinetics, biomarker changes, and potential indicators of tumor response through imaging and blood tests.

This clinical trial provides important insight into targeting MUC16 as a tumor-associated antigen (TAA) in solid cancers. While the 27T51 study uses CAR T-cell therapy to attack MUC16-positive tumor cells, CRISPR- based approaches in PDAC focus on reducing MUC16 expression at the genetic level to limit tumor growth, invasion, and immune evasion. Together, these strategies show us how CAR T-cell therapy seeks to destroy existing tumor cells by expressing an antigen targeted to MUC16, while CRISPR editing aims to suppress its production entirely. By learning more about the specificity of targeting MUC16 and the immune interactions observed in this trial, researchers can develop CRISPR-based MUC16 therapies specifically for PDAC that have the potential to revolutionize treatment, while reducing the inherent challenges of CAR T-cell therapy, such as off-target effects, antigen loss, or immune suppression.

While the clinical trial is still ongoing and thus the results have not yet been released, this study represents an important advancement in the application of CAR T-cell therapy to solid tumors, where success has usually been limited due to the immunosuppressive environment. By targeting MUC16, this trial seeks to determine whether 27T51 can become a safe and effective treatment to eradicate tumor cells in these aggressive cancers. If successful, this study could not only provide a new therapeutic option for patients with recurrent ovarian and related cancers, but it can also pave the way for adapting MUC16-targeted CAR T-cell therapy to other solid tumors, such as pancreatic cancer, that express similar antigens.

Conclusion

PDAC continues to be one of the most lethal cancers, with a five-year survival rate under 12% and limited effective treatment options for most patients. Continuous research has identified the large glycoprotein MUC16 as a major contributor in PDAC tumor progression, immune system evasion, and resistance to chemotherapy. CRISPR-Cas9, a revolutionary gene-editing tool, presents a promising solution to this problem, by directly suppressing or eliminating MUC16 expression. Studies in cell culture and animal models have shown encouraging results in reduction of tumor growth through CRISPR-based MUC16 targeting. By targeting MUC16 with a CRISPR-based therapy, there is potential that this novel treatment strategy will be further developed to increase treatments and survival outcomes for one of the most aggressive cancers.

Additionally, CRISPR-modified CAR-T cells targeting MUC16 show promise in other cancers, such as ovarian cancer, and present a potential pathway for similar use in PDAC. These findings suggest that CRISPR could be a critical component of future therapies against PDAC.

However, before doing so, several important limitations must be addressed. One of the major challenges facing CRISPR-based therapies is the potential for off-target effects, where

unintended genomic edits may cause harmful mutations or disrupt essential genes. While newer, more advanced Cas9 variants and improved guide RNA design have substantially reduced these risks, they have not been eliminated entirely (Wang et al., 2022). Moreover, the editing efficiency of CRISPR in solid tumors like PDAC remains inconsistent, which may affect the reliability of the therapy in clinical settings.

Another significant barrier is the method of delivery. Effective delivery of CRISPR components into PDAC tumor cells is particularly difficult due to the immune-evasive nature of the tumor. By targeting MUC16 with a CRISPR-based therapy, there is a hope that a novel treatment strategy will be developed to increase treatment and survival outcomes for one of the most aggressive cancers. While non-viral approaches such as lipid nanoparticles show potential for safety and adaptability, they often fall short in terms of efficiency (Integrated DNA Technologies, n.d.). In contrast, viral vectors can offer high efficiency but carry risks such as immune responses and insertional mutagenesis, making them less useful for repeated or long-term treatment.

Moving forward, several developments are critical for translating CRISPR-based MUC16 targeting into a viable treatment option for PDAC. These include enhancing delivery methods, improvising editing accuracy, and initiating early phase clinical trials to determine the safety and efficacy in human patients. Also, combinational approaches, such as pairing CRISPR with chemotherapy, immunotherapy, and CAR-T therapeutic strategies, could provide synergistic benefits.

In conclusion, while it is currently still in its early stages, the application of CRISPR to target MUC16 in pancreatic cancer shows a potential pathway towards fighting this disease. With continued research and innovation, this approach holds the potential to overcome hurdles in PDAC treatment and improve survival for patients facing one of the most aggressive forms of cancer.

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