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Review

Integrating CRISPR Technology with Key Genetic Markers in Pancreatic Cancer: A New Frontier in Targeted Therapies

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Abstract: Pancreatic cancer is the result of mutations in crucial genetic markers like *KRAS* and *TP53* that make treatment challenging. This article discusses how CRISPR Cas9 technology can be combined with these markers to create treatments. CRISPR allows for the alteration or repair of these mutations, with the aim of restoring gene function or blocking cancer-causing pathways. For instance, CRISPR has the potential to fix mutations in *TP53* or *CDKN2A* genes and restore *SMAD4* signaling or target the *KRAS* oncogene in the body's cells. However promising, it may be that CRISPR encounters obstacles like unintentional effects and challenges in effectively delivering it to pancreatic tumor cells. Furthermore, ethical concerns, especially related to the editing of the germline, need consideration. As techniques based on CRISPR advance, there is a chance for them to transform the treatment landscape for cancer by offering personalized therapies. More studies are needed to enhance how treatments are administered accurately and safely through methods and targeted testing for effectiveness.

Keywords: CRISPR-Cas9; pancreatic cancer; *KRAS*; TP53; CDKN2A; SMAD4; gene editing; targeted therapy; genetic mutations; personalized medicine

1. Introduction

Pancreatic cancer (PC), in particular, is worrisome because of its high mortality rate, as seen from the 64,050 cases and 50,550 deaths reported in 2023 alone [1]. The challenges revolve around the difficulty in detection and the limited effectiveness of existing chemotherapy treatments, which altogether make it rank as the third major cause of death due to cancer. Among the challenges in treating pancreatic cancer is the presence of complex genetic mutations that drive its progression [2,3]. The most notable mutations in Table 1 occur in genes like *KRAS*, *TP53*, *CDKN2A*, and *SMAD4*, all of which are pivotal in cell cycle regulation, apoptosis, and growth factor signaling pathways. *KRAS* mutations are found in over 90% of pancreatic cancer cases, contributing to uncontrolled cell proliferation, while *TP53*, *CDKN2A*, and *SMAD4* mutations interfere with tumor suppression and apoptotic pathways. Understanding these genetic drivers is critical, as it opens doors to targeted treatments that can attack the disease at its molecular core, potentially improving survival rates and treatment efficacy [4,5].

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Table 1. Key genetic mutations in pancreatic cancer.

Gene	Mutation Type	Role in Pancreatic Cancer	Implications for Treatment	References
KRAS	Oncogene, activating mutation	Promotes cell growth and proliferation	Targeting *KRAS* or its downstream pathways	[6–8]
TP53	Tumor suppressor, loss of function	Disrupts apoptosis and cell cycle arrest	CRISPR-based restoration of *TP53* function	[9–13]
CDKN2A	Tumor suppressor, deletion or mutation	Leads to loss in cell cycle control	CRISPR to restore or mimic *CDKN2A* function	[14–17]
SMAD4	Tumor suppressor, loss of function	Impairs TGF-β signaling and promotes metastasis	Restoration of *SMAD4* signaling pathways	[18–21]

Trustworthy biomarkers are vital for guiding the right therapy in cases like pancreatic cancer, where mutations in genes like *KRAS* and *TP53* fuel its aggressive behavior [5,22]. The obstacles we face today in the field and beyond have sparked curiosity in cutting-edge solutions such as the CRISPR/Cas system—a technology with great promise for precise genetic editing that could transform how we approach cancer treatment. CRISPR-Cas9 technology, a groundbreaking tool in gene editing, offers promising opportunities for targeted therapies in pancreatic cancer. First developed in the early 2000s, CRISPR's ability to make precise cuts in DNA sequences has revolutionized genetic research, enabling the targeted modification of specific genes [23]. In the context of pancreatic cancer, CRISPR holds the potential to directly target the genetic mutations driving the disease. For example, researchers are exploring CRISPR's ability to disrupt mutant *KRAS* or restore the function of *TP53*, *CDKN2A*, or *SMAD4*, thereby halting tumor growth or sensitizing cancer cells to chemotherapy. The goal of integrating CRISPR into pancreatic cancer treatment is to develop therapies that are more precise and personalized, minimizing damage to surrounding healthy tissues [24]. Some of the potential advances in CRISPR-based therapies have been organized in Table 2.

Table 2. Potential advances in CRISPR-based therapies for pancreatic cancer.

Therapy Type	CRISPR Approach	Target Gene	Potential Benefits	References
Gene Knockout	CRISPR-Cas9	*KRAS*	Inhibition of oncogenic *KRAS*	[25]
Gene Repair	CRISPR HDR	*TP53*, *CDKN2A*	Restoration of tumor suppressor function	[26,27]
Base Editing	CRISPR base editors	*TP53*	Correction of point mutations	[26,27]
Immune Cell Engineering	CRISPR-modified T cells	Various	Enhancing T-cell recognition of tumor cells	[28]

2. CRISPR Technology: Mechanisms and Applications

CRISPR-Cas9 technology (Figure 1) is a revolutionary tool for genome editing, enabling precise and targeted modifications to DNA sequences. The system consists of two main components: the Cas9 enzyme and a guide RNA (gRNA). The gRNA is designed to

recognize a specific DNA sequence, while Cas9 acts as molecular scissors to cut the DNA at this targeted location [23]. Once the DNA is cut, the cell's natural repair mechanisms take over, either through non-homologous end joining (NHEJ) or homology-directed repair (HDR). These mechanisms can be harnessed to introduce desired mutations, delete specific gene sequences (knockout), insert new genes (knock-in), or make base-level changes without introducing a double-stranded break (base editing). In cancer treatment, gene knockouts can disable oncogenes, knock-ins can restore tumor suppressor genes, and base editing allows for the precise correction of point mutations. These various CRISPR strategies hold immense potential for addressing the genetic mutations driving cancer progression, including those in pancreatic cancer [29,30].

CRISPR technology has greatly advanced cancer research by enabling functional genomic screens that help identify genes essential for tumor growth and survival. These screens involve knocking out thousands of genes across cancer cell lines to observe their effects on cellular behavior, allowing researchers to pinpoint potential therapeutic targets. In the context of pancreatic cancer, CRISPR-based models have been used to study the roles of key mutations like *KRAS* and *TP53* in cancer initiation and metastasis. For instance, researchers can introduce mutations found in human pancreatic tumors into animal models, allowing for a more accurate representation of human disease. This approach provides invaluable insights into how specific genetic alterations contribute to the aggressive nature of pancreatic cancer and how targeting these mutations might affect treatment response [31].

Despite its promise, the translation of CRISPR from the lab to the clinic faces several hurdles. Currently, there are several CRISPR-based therapies under investigation, including treatments for blood disorders like sickle cell disease and cancers such as leukemia and lymphoma. These therapies typically involve ex vivo gene editing, where patient cells are modified outside the body and then reintroduced. In pancreatic cancer, however, the development of in vivo CRISPR therapies—where editing occurs directly in the patient's body—remains in the early stages. Major challenges include delivery methods, off-target effects, and immune responses triggered by the CRISPR components. While lipid nanoparticles and viral vectors are being explored as delivery vehicles, ensuring precision and minimizing unintended mutations are critical for successful clinical applications. Furthermore, ethical concerns and regulatory hurdles continue to shape the pace at which CRISPR technologies will move from bench to bedside [32].

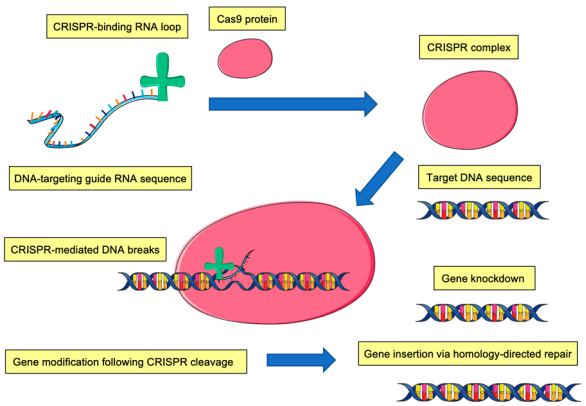

Figure 1. In the CRISPR/Cas system setup shown in Figure 1, the guide RNA (gRNA) is specifically created to aim at a gene. Then, it attaches to the Cas protein to form the CRISPR complex. The gRNA features a loop structure that aids in its connection to Cas. Following the formation of this complex, it triggers a double-strand break in the desired gene. In the case where there is a donor template, gene insertion can occur. In cases where the DNA is damaged and requires repair, it often leads to a gene becoming nonfunctional. This can result in what is known as gene knockdown [33].

3. Targeting *TP53* with CRISPR

The *TP53* gene, often referred to as the "guardian of the genome", is responsible for regulating cell cycle arrest, DNA repair, and apoptosis in response to DNA damage. The loss of *TP53* activity allows cells to bypass apoptosis, which in turn leads to unchecked cellular proliferation and the accumulation of additional genetic abnormalities, further exacerbating cancer development. The disruption of *TP53* also poses a significant challenge for treatment, as tumors with these mutations often exhibit resistance to chemotherapy and radiation therapy. Current research is focused on restoring wild-type *TP53* function or targeting downstream pathways affected by its loss to reintroduce control over the cell cycle. *TP53* mutations, present in 50–90% of PDAC, play a significant role in carcinogenesis, prognosis, and treatment response [9–12]. Located on chromosome 17, *TP53* functions as a tumor suppressor by regulating cell division. Mutations in *TP53*, which are critical in cancer pathogenesis, lead to uncontrolled cell growth. These mutations, primarily missense and truncating variants, disrupt mRNA degradation mechanisms. Gain-of-function (GOF) *TP53* mutations, in particular, promote tumor proliferation and chemotherapy resistance and often co-occur with *KRAS* mutations, indicating early *KRAS* involvement in pancreatic carcinogenesis [11,13].

Recent advancements in pancreatic cancer research have leveraged CRISPR-Cas9 technology to explore molecular mechanisms and potential therapeutic targets, particularly in relation to *TP53* mutations, chemoresistance, and immune suppression. One study utilized LASSO-Cox regression to identify a five-gene signature linked to *TP53* mutation status. High-risk patients exhibited increased tumor mutation burden, enhanced presence of immunosuppressive cells, and poorer survival rates [10]. Within the TIDE framework, CRISPR screens further evaluated these genes' roles in immune response, underscoring the utility of CRISPR for functional validation. In vivo CRISPR/Cas9-mediated somatic recombination has been crucial for developing genetically engineered mouse models (GEMMs) of PDAC. Using adeno-associated virus (AAV) vectors, genes like *KRAS*, *TRP53*, *LKB1*, and *ARID1A* were targeted, generating precursor lesions that progress to PDAC, demonstrating CRISPR's efficiency in modeling disease progression [34]. A bioinformatics tool called PAMfinder was employed to identify cancer-specific protospacer adjacent motifs (PAMs) in pancreatic tumors, allowing for selective CRISPR-Cas9 targeting, minimizing off-target effects, and inducing selective PDAC cell death [35]. Increased CRISPR-Cas9 target sites have been associated with greater cytotoxicity due to chromosomal instability and polyploidization, emphasizing the complexity of CRISPR-induced cytotoxicity [36]. Researchers also used CRISPR to explore chemoresistance mechanisms, identifying that ABCG2 activation conferred multi-drug resistance, while HDAC1-mediated epithelial-to-mesenchymal transition (EMT) was implicated in resistance to chemotherapy drugs [37]. CRISPR/Cas9 technology is gaining traction for cancer therapies, including addressing *TP53* mutations. Emerging tools like base editors and prime editors provide precise gene corrections without double-strand breaks (DSBs), holding promise for *TP53* mutation correction. CRISPR/dCas9 tools are also being explored for regulating gene expression and epigenetic modifications [26].

Researchers have leveraged CRISPR technology to uncover key regulators of *p53* stability, identifying *FBXO42* as a positive and *C16orf72/HAPSTR1* as a negative regulator [38]. Introducing the *p53R172H* mutation in pancreatic ductal adenocarcinoma (PDAC) models led to increased neutrophil infiltration and reduced T-cell presence. Lowering CXCL2 levels reduced neutrophil infiltration and improved therapy efficacy, illustrating the role of *p53R172H* in immune suppression and resistance [39]. Genome-wide CRISPR screens revealed that TP53-associated toxicity is heightened in regions with active chromatin, with chromatin context influencing the repair pathway choice and DNA double-strand break

(DSB) toxicity [40]. The *p53 R248W* mutation, known for stabilizing *p53* through Hsp90, enhances cell migration by deregulating the STAT3 pathway, driving cancer progression. CRISPR strategies could help further dissect the role of specific p53 mutations in cancer [41]. Three target proteins—RUVBL1, HSPA9, and XPO1—were identified as essential for cancer cell survival, offering potential therapeutic targets [42]. A bispecific antibody targeting the *p53R175H* mutation was developed to activate T cells and selectively kill cancer cells, with CRISPR validating its specificity [43]. In vivo CRISPR screening revealed the importance of the terpenoid backbone biosynthesis pathway for PDAC growth, suggesting therapeutic potential in targeting lipid metabolism [44]. In a KrasG12D-driven mouse model, Nutlin-3a activation of p53 suppressed metaplasia and tumorigenesis, while CRISPRi downregulation of p53 reduced differentiation markers [45]. Additionally, the CRISPR knockout of *Trp53* amplified the aggressive traits of PDAC cells in acidic tumor microenvironments [46]. CRISPR/Cas9 has accelerated cancer model creation and enabled in vivo genetic screens for novel cancer drivers and therapeutic targets [47].

Key mutations, including *KRAS*, *TP53*, *CDKN2A*, and *SMAD4*, drive pancreatic cancer progression. Genetically engineered mouse models (GEMMs) are crucial for studying these mutations, with inducible systems enabling precise temporal and spatial gene expression. The combination of CRISPR/Cas9 with other systems like Cre-loxP has further advanced PDAC modeling by introducing mutations such as *KRASG12D* and *TP53R172H*, which closely replicate human PDAC [48]. Researchers developed a library of 39 patient-derived PDAC organoids to explore cancer progression, revealing that loss of *Wnt* dependency occurs during malignant transformation. CRISPR was used to introduce mutations in driver genes, illustrating tumor heterogeneity [49]. Genome-wide CRISPR screens identified *PKMYT1* as a therapeutic target; its inhibition disrupted cell cycle progression and induced apoptosis, suggesting potential treatment strategies [50]. Overexpression of *ASAP2*, via increased DNA copy numbers, promotes PDAC growth through EGFR signaling. CRISPR knockout of *ASAP2* revealed that niclosamide, an antiparasitic drug, could suppress PDAC growth, showing potential for drug repurposing [51]. Targeting *MUC4*, a mucin glycoprotein, delayed pancreatic lesion formation in a conditional knockout mouse model, suggesting that inhibiting *MUC4-EGFR* interactions may slow PDAC progression [52].

4. CDKN2A and CRISPR-Based Interventions

Discovered in 1994, the *CDKN2A* gene, which encodes the cell-cycle inhibitor p16, is frequently mutated in pancreatic cancers [14]. As a crucial tumor suppressor located on chromosome 9, *CDKN2A* produces proteins like *p16(INK4A)* and *p14(ARF)*, which are key regulators of various cancer-related processes. These proteins inhibit cell-cycle progression by binding to and blocking cyclin-dependent kinases CDK4/6, keeping the retinoblastoma (Rb) protein active and preventing the transition from the G1 to S phase of the cell cycle [15,16]. Moreover, *CDKN2A* promotes apoptosis and senescence, suppresses cancer-associated mechanisms such as cell-in-cell structure formation and anchorage-independent growth, and modulates anti-tumor immunity by affecting immune-cell infiltration. *CDKN2A* dysregulation, often seen through genetic and epigenetic changes, is a common hallmark of various cancers, leading to unchecked cell proliferation and survival [17]. By encoding tumor suppressor proteins like *p16(INK4A)* and *p14(ARF)*, *CDKN2A* controls cell growth by inhibiting cyclin-dependent kinases, which prevents excessive cell division and promotes senescence and apoptosis. This gene is associated with numerous cancers, including brain tumors, melanoma, and lung cancer. On chromosome 9, *CDKN2A* plays an essential role in curbing cell proliferation and invasion in many cancers. Its dysfunction, therefore, is a critical factor in cancer development [15,16].

A genome-wide CRISPR screen identified *RAB10* as a synthetic lethal gene in colorectal and pancreatic cancers with *SMAD4* loss. Knocking out *RAB10* significantly reduced cell viability in *SMAD4*-deficient cells and reintroducing it restored survival, suggesting *RAB10* as a potential therapeutic target for cancers lacking *SMAD4*, often resistant to conventional therapies [53]. In another study, researchers investigated combination therapies for *KRAS*-mutant PDAC, combining *CDK4/6* inhibitors with *ERK-MAPK* inhibitors. This approach synergistically suppressed tumor growth by preventing compensatory upregulation of *ERK*, *PI3K*, anti-apoptotic signaling, and *MYC* expression. A Phase I clinical trial is underway to test this combination, with CRISPR-Cas9 screens identifying pathways like *PI3K-AKT-mTOR*, cell-cycle regulation, and DNA damage repair as key modulators of treatment sensitivity [54]. A novel method for generating genetically engineered mouse models (GEMMs) of PDAC using CRISPR/Cas9 and AAV-delivered guide RNAs has also been developed. This in vivo somatic recombination approach allows for the rapid and cost-effective creation of complex PDAC models, accurately mimicking the genetic intricacies of human PDAC, including precursor lesions and invasive cancers [55]. Another study examined the role of the CDKN2A gene, a tumor suppressor, in pancreatic development and cancer. Using hiPSCs and pancreatic organoids, researchers found that *CDKN2A* loss accelerates dysplasia and metastasis in *KRAS-mutant* PDAC. While CRISPR was not directly involved, gene-editing and stem cell technologies were central, suggesting future CRISPR applications could enhance model precision for personalized medicine [56]. A final study used inducible in vivo CRISPR-Cas9 to create focal mutations and large deletions in pancreatic tissue. Disrupting genes like *Rnf43* and *CDKN2A* generated complex alleles, better reflecting human cancer genetics. Loss of *Rnf43* accelerated Kras-driven tumorigenesis, demonstrating CRISPR's potential to develop sophisticated PDAC models essential for precision oncology [57].

A study utilized CRISPR knockout screening to identify drug targets and predict responses in PDAC. By combining large-scale in vivo and in vitro CRISPR screens, researchers pinpointed genes whose loss increased the efficacy of *MEK* signaling inhibitors, such as *CENPE* and *RRM1*, both involved in cell cycle regulation. This led to the development of DREBIC, a method that combines CRISPR viability scores and gene expression data to predict drug responses, underscoring CRISPR's role in enhancing cancer therapies [58]. Another study explored the role of *CDK4/6* inhibition in PDAC, focusing on the combination of the *CDK4/6* inhibitor PD-0332991 and miR-21 knockout using CRISPR/Cas9. This combination significantly reduced cell viability, induced apoptosis, and caused cell cycle arrest in PDAC cells. The study revealed that miR-21 knockout synergistically increased the effectiveness of PD-0332991, suggesting a novel strategy for PDAC treatment [59].

Genomic characterization of PDAC identified key genetic aberrations that could be targeted for improved therapeutic outcomes. *CDKN2A* homozygous deletions, often co-occurring with *MTAP* deletions, were linked to synthetic lethality and tumor metabolism alterations. High-level MYC amplifications and a pancreas-specific super-enhancer region associated with MYC were identified, offering potential biomarkers and therapeutic targets for PDAC [60]. A comprehensive review of PDAC genetics emphasized the role of mutations in *KRAS*, *TP53*, *SMAD4*, and *CDKN2A,* highlighting the importance of genome-wide association studies (GWAS) in identifying high-risk individuals and novel therapeutic targets. The review discussed the utility of biological models, such as patient-derived xenografts (PDX), organoids, and organ-on-chip systems, which better mimic the tumor microenvironment and aid in studying disease progression and drug responses. CRISPR technology is essential in developing genetically engineered mouse models (GEMMs) and organoids for PDAC research [61].

The tumor suppressor *ISL2* was identified through in vivo CRISPR screening as a key regulator of PDAC growth. *ISL2* silencing promoted aggressive tumor growth, reprogramming cells to favor oxidative phosphorylation (OXPHOS) over glycolysis. Reintroduction of *ISL2* or CRISPR-mediated upregulation reduced tumor growth, suggesting that targeting mitochondrial metabolism could offer therapeutic potential [62]. Another study focused on the regulation of the *CDKN2A/B* locus, revealing that TGFβ signaling selectively induces ARF expression through a novel enhancer region. CRISPR-Cas9 editing of this enhancer disrupted its ability to regulate *ARF* and *INK4B* expression, highlighting the potential of targeting regulatory elements to offer new therapeutic strategies [63]. Finally, a review of CRISPR/Cas9 technology emphasized its transformative impact on cancer research. CRISPR enables precise genome editing, aiding in the identification of therapeutic targets and creating accurate in vivo cancer models. The review also discussed the potential of CRISPR-based tools, like CRISPRa and CRISPRi, and the challenges of off-target effects and ethical considerations that need to be addressed for clinical adoption [64].

A study investigated the use of CDK4/6 inhibitors in PDAC treatment, finding that sequential treatment—applying inhibitors after chemotherapy—enhanced the efficacy of chemotherapy by preventing cell recovery from chromosomal damage. This approach was effective in mouse models and patient-derived xenografts, suggesting improved outcomes for PDAC patients [65]. Another study combined CDK4/6 and ERK inhibitors, effectively reducing PDAC growth, while CRISPR screens revealed that inhibiting genes in the *PI3K-AKT-mTOR* pathway enhanced the efficacy of CDK4/6 inhibitors. Further research used a CRISPR-Cas9 loss-of-function screen to identify genes that enhance the efficacy of CDK4/6 inhibitors in pancreatic cancer. The combination of ERK and CDK4/6 inhibitors showed synergistic effects, significantly reducing tumor cell proliferation in both cell lines and patient-derived organoids [66].

FAM110C, a potential tumor suppressor gene that is frequently silenced in PDAC, has been studied. CRISPR-Cas9 knockout of *FAM110C* increased PDAC cell proliferation and invasion, while its re-expression reduced malignancy. Targeting the ATR/CHK1 pathway may benefit patients with FAM110C-deficient tumors [67]. Research using CRISPR/Cas9 also explored epigenetic regulation in PDAC, distinguishing two molecular subtypes: classical and basal. The classical subtype showed active histone acetylation, while the basal subtype had repressive marks. Monotherapy with histone inhibitors was ineffective, but multiplex CRISPR targeting multiple epigenetic regulators led to cell death, highlighting the complexity of epigenetic targeting [68].

5. Addressing SMAD4 Loss-of-Function with CRISPR

SMAD4 plays a crucial role in the TGF-β signaling pathway, regulating cellular activities such as growth, differentiation, apoptosis, and migration. Upon TGF-β binding to receptors, *SMAD* proteins form a complex with *SMAD4*, which then moves to the nucleus to regulate gene expression. *SMAD4* maintains pathway sensitivity through feedback mechanisms, activating receptor-regulated *SMADs* like *SMAD3* and *SMAD1* [18,19]. Beyond *TGF-β*, *SMAD4* interacts with the Wnt signaling pathway, regulating genes such as *FZD4*, highlighting its broad regulatory role [20]. In cancer, *SMAD4* is essential for inducing cell-cycle arrest and apoptosis, helping control cell proliferation, and regulating EMT (epithelial–mesenchymal transition), a process involved in cancer progression and metastasis [18]. *SMAD4* dysregulation is linked to autophagy, invasion, and metastasis, emphasizing its role in disease development [20]. Moreover, *SMAD4* has feedback regulatory functions within the TGF-β pathway. For instance, reducing *SMAD4* expression decreases *TGFBR2* mRNA, while overexpressing *SMAD4* increases it [21]. Its inactivation is common in over half of PDAC cases and other cancers, often accelerating progression

when combined with mutations in oncogenes like *KRAS* or *APC*. Though *SMAD4* loss typically does not initiate tumors, in skin cancer, it can impair DNA damage response, directly contributing to tumor initiation [18].

The research explores multiple genetic targets and mechanisms associated with *SMAD4-deficient* pancreatic ductal adenocarcinoma (PDAC) and colorectal cancers. *SMAD4*, a key tumor suppressor, is frequently mutated in these cancers, leading to poor prognosis and treatment resistance. Using CRISPR-Cas9 screens, *RAB10* was identified as a synthetic lethal gene in *SMAD4-deficient* pancreatic cancers, where *RAB10* knockout reduced proliferation specifically in *SMAD4-negative* cells [53]. *ASAP2*, an *ArfGAP* family member, was also identified as a driver gene in PDAC, with elevated *ASAP2* expression linked to poor prognosis. CRISPR-Cas9 knockout experiments demonstrated that *ASAP2* promotes tumor growth via EGFR pathway activation, facilitating cell cycle progression [51]. In an effort to develop gene-editing models for PDAC, researchers used adeno-associated virus (AAV) to deliver CRISPR/Cas9 to induce mutations in the pancreas of wild-type mice. Two Cas9 orthologs, Streptococcus pyogenes Cas9 (SpCas9) and Campylobacter jejuni Cas9 (CjCas9), were utilized, effectively inducing double-strand breaks in PDAC-related genes. Both SpCas9 and CjCas9 induced pancreatic intraepithelial neoplasia (PanIN) and early markers of pancreatic cancer progression, demonstrating the utility of CRISPR-based gene editing for modeling PDAC and studying therapies [69]. Additionally, *HNF4G* overexpression, driven by SMAD4 deficiency, was found to promote PDAC cell migration and metastasis. Metformin, a diabetes drug, inhibits HNF4G through *AMPK-mediated* phosphorylation, making it a potential therapeutic option [70]. In another discovery, *SMAD4-loss* increased tumor immunogenicity by enhancing STING-mediated type I interferon signaling, which improved MHC-I expression and activated CD8+ T cells, resulting in better tumor control in immunocompetent mice [71].

Another study also identified a super-enhancer regulating *TGFBR2* expression in pancreatic cancer cells, with CRISPR-Cas9 deletion of the enhancer impairing TGF-β signaling, EMT, and cancer cell migration. The BRD4 inhibitor JQ1 confirmed the enhancer's role in regulating *TGFBR2* and *EMT* [72]. Resistance mechanisms in *SMAD4-deficient* PDAC included increased resistance to complex I inhibitors via enhanced mitophagy driven by MAPK/ERK signaling [73]. Additionally, *PGK1* was upregulated, supporting *EMT* and metastasis, and its nuclear translocation repressed E-cadherin. Targeting *PGK1* could serve as a therapeutic strategy [74]. Moreover, *ID1* was identified as critical for PDAC progression by preventing TGF-β-induced apoptosis, and its suppression reduced tumor growth in mouse models [75]. Lastly, novel *SMAD4* nonstop mutations, leading to its degradation, were discovered in tumors with low mutation burdens [76].

In SMAD4-deficient pancreatic ductal adenocarcinoma (PDAC), a transcription factor complex involving *NFATc1*, *SMAD3*, and *cJUN* promotes the expression of *RRM1* and *RRM2*, which confer resistance to gemcitabine by competing for DNA incorporation. Inhibition of MAPK signaling disrupts this complex, reducing *RRM1* and *RRM2* levels, thereby sensitizing cancer cells to gemcitabine and significantly reducing tumor growth in *SMAD4-deficient* models [77]. Research also revealed that *RAC1B inhibits* TGF-β1-induced cell migration and epithelial–mesenchymal transition (EMT) by downregulating *ALK5*. Loss of *RAC1B* leads to increased *ALK5* expression, enhancing *SMAD3* activation and promoting migration and *EMT*. Suppression of *ALK5* through RNA interference or pharmacological inhibition mitigated the effects of *RAC1B* loss, emphasizing its role in TGF-β1 signaling [78]. Additionally, *SMAD2* and *SMAD3* promote cell migration in the absence of *SMAD4*, with CRISPR/Cas9 knockout of *SMAD2/3* reducing migration and invasion. Active *SMAD2/3* in *SMAD4-deficient* cells correlated with increased migration and poor clinical outcomes in patients [79].

A review highlighted the challenges of early PDAC diagnosis and explored emerging technologies like nanomedicine and CRISPR/Cas9 for improved diagnosis and treatment, emphasizing targeting molecular pathways such as *KRAS* and *PI3K/AKT* [80]. *RAC1B* is inversely correlated with PDAC metastasis and enhances *SMAD3* expression while inhibiting migration. Biglycan (BGN) was identified as a downstream mediator of this pathway [81]. In vivo CRISPR screening revealed *KDM3A* as an epigenetic regulator affecting the immune environment in PDAC, suggesting that its loss improves immune infiltration and sensitivity to immunotherapy [82]. The CRISPR-CasRx system effectively silenced the mutant *KrasG12D* transcript in PDAC cells, reducing tumor growth and enhancing gemcitabine sensitivity [83]. Lastly, inhibiting *ALK2* increased PDAC cell migration, revealing a negative feedback loop between *ALK2* and *RAC1B* that regulates migration in PDAC [84].

6. CRISPR Approaches for KRAS Mutations

KRAS is an oncogene mutated in over 90% of pancreatic cancers, making it a crucial driver of the disease's aggressiveness. The *KRAS* gene encodes a GTPase protein that, when activated, stimulates cell growth and division through signaling pathways such as MAPK/ERK and PI3K/AKT. Mutations in *KRAS* lock the protein in an active state, leading to constant activation of these pathways and unchecked cellular proliferation. Despite its critical role in pancreatic cancer, directly targeting *KRAS* mutations has proven difficult due to the protein's structure, which lacks suitable binding pockets for drugs. Recent advancements, including *KRAS G12C* inhibitors, provide some hope, though these therapies only target a small subset of *KRAS* mutations, and tumors often develop resistance to them. *KRAS* mutations are highly prevalent in pancreatic tumors, with 90–95% of pancreatic adenocarcinomas harboring mutations, making it the most commonly mutated gene in this cancer type [85]. These mutations occur in 84% of all RAS-mutant cancers, and nearly 95% of PDAC exhibit *KRAS* mutations, highlighting PDAC as one of the most RAS-dependent cancers. The most frequent mutations occur in codons 12, 13, and 61, with G12D being the most common substitution. However, despite their high prevalence, few *KRAS* mutations are actionable, presenting challenges for targeted therapies [6,7].

KRAS (Kirsten rat sarcoma viral oncogene homolog) mutations are present in up to 25% of all human tumors, making it one of the most frequently activated oncogenes. Recent studies have shown that the presence of *KRAS* mutations can influence treatment decisions in cancer patients [8]. In PDAC, oncogenic *KRAS* plays a pivotal role in tumor initiation and progression by promoting the generation of reactive oxygen species (ROS) through metabolic alterations, activating key signaling pathways involved in the development of the disease [86]. A study found that blocking TGF-β signaling in the pancreas, alongside active *KRAS* expression, resulted in aggressive PDAC with increased progression, metastasis, and invasion [87].

The progression of pancreatic cancer, particularly from intraepithelial neoplastic lesions to adenocarcinoma, is significantly driven by early *KRAS* mutations. The interplay between *KRAS* and EGFR signaling emphasizes the necessity for a thorough understanding of these pathways in developing effective therapeutic strategies and personalized treatments [88]. A genomic analysis of 456 pancreatic tumors, mainly PDAC, identified 32 significantly mutated genes across 10 molecular pathways, with *KRAS* mutations present in 92% of cases. This study also highlighted recurrent copy number alterations involving genes like *MET* and *CDKN2A* [89].

Another investigation into lipid metabolism revealed a connection between oncogenic *KRAS* and increased fatty acid storage in intracellular lipid droplets. This storage was facilitated by the suppression of hormone-sensitive lipase (HSL) by *KRAS*, contributing to tumor cell invasion [90]. High RAS activity, coupled with the loss of tumor suppressors, are critical drivers of PDAC development [91]. In a notable study, three pancreatic cancer cell lines containing the

KRASG12D mutation were used to explore the effects of CRISPR/Cas9. Researchers specifically targeted and knocked out the *KRASG12D* mutation while preserving the wild-type *KRAS* gene. Although this approach did not result in complete cell growth arrest or apoptosis, it did reduce cancer cell proliferation, suggesting that PDAC cells might employ alternative survival pathways when *KRAS* is inactivated. RNA sequencing of one cell line identified 417 differentially expressed genes, revealing significant downregulation of genes associated with cell migration and proliferation, indicating potential therapeutic targets [25].

Another innovative approach utilized exosomes, engineered from HEK293T cells, to deliver CRISPR/Cas9 constructs targeting *KRASG12D*. This method effectively reduced *KRASG12D* expression and suppressed tumor proliferation in mouse models, presenting a safer alternative to viral vectors for CRISPR delivery [92]. The researchers also experimented with an RNA-targeting CRISPR system, CRISPR-Cas13a, achieving a 94% knockdown efficiency of *KRAS-G12D* mRNA. This led to significant inhibition of PDAC cell proliferation and apoptosis, as well as blocking critical oncogenic pathways such as AKT and ERK. In vivo, CRISPR-Cas13a treatment significantly reduced tumor growth, highlighting its therapeutic potential against KRAS-driven cancers [93]. Another approach used a capsid-optimized adenovirus-associated virus 8 (AAV8) vector to deliver the CRISPR-CasRx system into PDAC tumors, including patient-derived tumor xenografts (PDX). CRISPR-CasRx, guided by a KrasG12D-specific gRNA, precisely silenced the mutant transcript without off-target effects, suppressing downstream Akt and Erk pathways. Importantly, this approach not only inhibited tumor proliferation but also enhanced the sensitivity of the cancer cells to gemcitabine, a common chemotherapy drug. The combination treatment significantly inhibited tumor growth in mouse models, showing potential for clinical application. The study concluded that CRISPR-CasRx could effectively and safely target the *KRASG12D* mutation in pancreatic cancer [83,94]. A study on KRAS knockout in a mouse model revealed that although *KRAS* is essential for tumor initiation, some PDAC cells can survive its loss, particularly in immunodeficient environments. The research indicated that *KRAS* mutations facilitate immune evasion, with *BRAF* and *MYC* identified as mediators of this immune suppression [95]. Recent research has successfully demonstrated the knockout of the *KRASG12D* mutation in various cell lines, confirming the complexity and varied impact of its elimination on signaling pathways [25]. Lastly, a review highlighted the potential and challenges of CRISPR technology in targeting KRAS-driven cancers, emphasizing the need for efficient delivery systems and the exploration of downstream pathway targeting as alternative therapeutic strategies [96].

A novel approach using exosomes to deliver CRISPR/Cas9 has been proposed to specifically target the *KRASG12D* mutation, a common driver in pancreatic cancer. Unlike traditional methods like viral vectors, which carry risks such as immunogenicity and toxicity, exosomes offer a more efficient and less immunogenic alternative. The exosome delivery system demonstrated success in knocking down the *KRASG12D* mutation, leading to suppressed cancer cell proliferation and tumor growth in both in vitro and in vivo models. This method significantly reduced tumor size and impaired oncogenic signaling pathways, offering promise as a targeted therapeutic option for KRAS-driven cancers. However, further optimization is needed to improve delivery efficiency and ensure long-term safety for clinical applications [92]. *KRAS* mutations play a critical role in pancreatic cancer, impacting diagnosis, prognosis, and treatment strategies. While *KRAS* mutation assays are established for cancers like lung and colorectal cancer, their clinical use in pancreatic cancer is still evolving. Targeting *KRAS* has been challenging due to its strong affinity for GTP and the lack of accessible binding sites. However, recent advances in CRISPR-Cas9 technologies and small-molecule inhibitors offer new opportunities for developing treatments aimed at *KRAS* mutations [97].

Using CRISPR-Cas9 technology, researchers examined the effects of variations in *KRAS* expression on PDAC progression and metastasis. Increased dosage of the *KRASG12D* mutation was associated with both early tumorigenesis and metastasis. By employing CRISPR-Cas9 mutagenesis in mouse models, they traced the evolutionary pathways of *KRAS*-driven cancers and observed how different levels of *KRAS* expression resulted in distinct cancer subtypes with varied metastatic potential [98]. In another approach, researchers used retrograde pancreatic ductal injection to deliver viral vectors carrying Cre recombinase and guide RNAs for CRISPR-Cas9. This allowed them to induce genetic alterations in adult mice, targeting *Lkb1*, a tumor suppressor mutated in PDAC. Combining CRISPR-Cas9 with *KRAS* mutations led to the development of pancreatic tumors that closely mimicked human PDAC, offering insights into how specific mutations contribute to tumor progression [99]. Lastly, researchers explored CRISPR-Cas9-mediated knockouts of the mutant *GNAS* allele, comparing the behavior of mutant and wild-type cells. They found that *GNAS* mutations reduced tumor invasiveness, whereas wild-type *GNAS* increased tumor growth. Transcriptome analyses showed that *GNAS* mutations suppressed NOTCH signaling, highlighting the dual role of *GNAS* in pancreatic cancer. This research suggests that inhibiting *GNAS* may increase tumor aggressiveness [100]. Furthermore, CRISPR-Cas9 loss-of-function screens identified *ITGB1* as a key modulator of response to *KRAS* inhibitors, particularly in combination therapies targeting the EGFR, PI3K, and MTOR pathways, which enhanced therapeutic outcomes [101].

Researchers have employed the CRISPR-Cas9 system to selectively target *KRAS* mutations such as *KRASG12V*, *KRASG12D*, and *KRASG13D* without affecting the wild-type *KRAS* allele. Through a reporter assay, specific guide RNAs (gRNAs) were identified and validated, leading to the disruption of mutant *KRAS* in cancer cell lines. This inhibited cancer cell proliferation and tumor growth while sparing wild-type cells [102]. Another study introduced DNA double-strand breaks (DSBs) in *KRAS* mutations at codon 12, effectively disrupting the mutated allele and inhibiting cancer cell proliferation. This approach led to reduced cell viability and tumor growth [103]. However, long-term Cas9 expression led to escape variants, which were addressed using adenine base editors (ABEs) to correct specific *KRAS* and *TP53* mutations. Base editing successfully depleted mutant cells without inducing escape variants, showing promise in personalized cancer treatment [104].

Figure 2 below diagrammatically summarizes the genes mentioned in the main text with appropriate references.

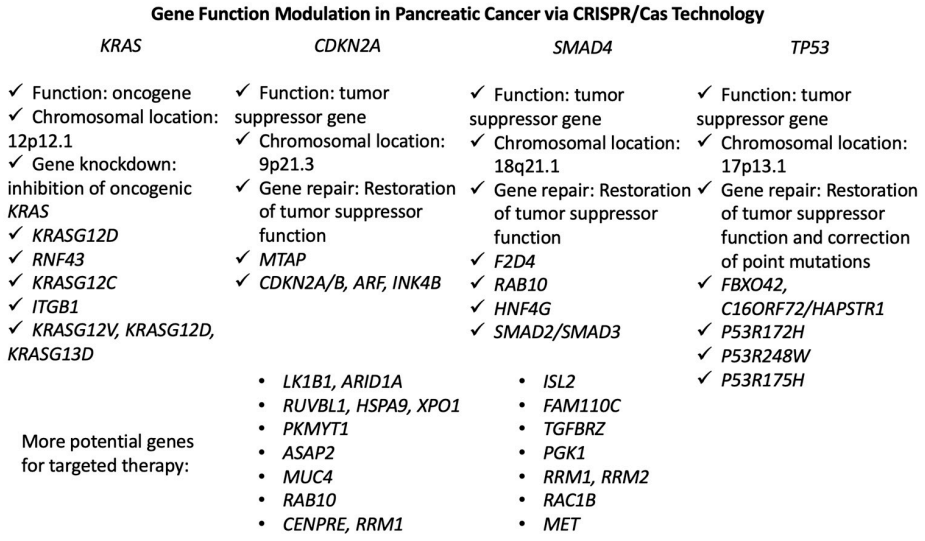

Figure 2. Schematical presentation of the relation between CRISPR/Cas9 and pancreatic cancer in the context of PC genetic gene markers.

7. Challenges, Future Directions, and Ethical Considerations

The use of CRISPR technology in treatments shows potential but encounters considerable technical hurdles when it comes to addressing diseases such as pancreatic cancer specifically. A primary concern revolves around off-target effects caused by CRISPR inadvertently triggering mutations in locations other than the intended target area. This unintended editing can result in outcomes such as interfering with genes or triggering cancer-causing genes that may worsen the patient's health condition. Using CRISPR for gene editing presents challenges due to variations in efficiency based on mutation types and cell types; achieving outcomes across tissues is hard to accomplish as a result of these differences. Delivery methods are also a significant obstacle. The dense stromal environment and aggressive nature of pancreatic cancer cells make it tough to introduce CRISPR components (Cas9 and guide RNA) into them. Different techniques for delivering treatments involve vectors and lipid nanoparticles. However, each approach comes with its challenges, like triggering reactions or facing obstacles in effectively targeting specific cancer cells [32,105–107].

CRISPR treatments for cancer have the potential to work alongside therapies like chemotherapy and immunotherapy by targeting the genetic factors that drive tumor resistance and boosting treatment effectiveness. For example, using CRISPR Cas9 to modify genes like *KRAS* and *TP53*—mutations in cancer—could potentially reverse drug resistance or make tumors more receptive to chemotherapy. When combined with immunotherapy, CRISPR might boost the body's response by altering tumor cells to show antigens or reducing checkpoint molecules, which could improve the effectiveness of immunotherapy. One way to enhance the systems response against cancer is by pairing CRISPR technology with immune checkpoint inhibitors to counteract the environment in tumors and improve the body's ability to target and eliminate cancer cells effectively. In the future, integrating CRISPR techniques with treatments could lead to efficient therapy plans [108–110].

Using CRISPR on cancer cells poses difficulties because of the genetic intricacies and variations within the disease itself. Pancreatic cancer growths frequently contain mutations like those found in genes such as *KRAS*, *TP53*, *CDKN2A,* and *SMAD4,* creating challenges in pinpointing an effective treatment target. Moreover, the surrounding pancreatic cancer tumors are characterized by a stroma that serves as a physical obstacle, impeding the delivery of CRISPR components to cancerous cells. One more obstacle is the fact that pancreatic cancer cells display flexibility, which enables them to adjust and build resistance against treatments like gene editing. Consequently, "even if CRISPR effectively modifies the targeted gene, the cancer might transform over time, requiring a combination of treatments or utilizing CRISPR along with therapy options, like immunotherapy or chemotherapy" [111].

The ethical implications of employing CRISPR in treatments are significant when considering the possibility of genome editing in reproductive cells like sperm and eggs that may affect future generations with unforeseen consequences and long-lasting effects due to uncertainties about potential unintended outcomes beyond the targeted edits. One important ethical concern involves obtaining consent when providing care to groups such as individuals with cancer diagnoses. It is crucial that patients grasp the nature of treatments based on CRISPR technology and comprehend the associated risks and possible unforeseen consequences in the term. Furthermore, the regulations governing CRISPR-based therapies are continuously developing; some nations implement prohibitions on editing genes in germline cells, while others adopt a lenient approach. We require defined and uniform regulations at a level to guarantee the responsible and ethical use of CRISPR in medical environments. This is especially crucial as the technology transitions from laboratory experiments to trials involving humans [107,112].

8. Conclusions

The combination of CRISPR technology with indicators in pancreatic cancer like *KRAS*, *TP53*, *CDKN2A*, and *SMAD* provides exciting new opportunities for tailored treatments. By modifying these genes, CRISPR has the potential to address existing treatment constraints; however, there are challenges to overcome in the areas of delivery techniques and minimizing unintended effects. Despite these hurdles, therapies utilizing CRISPR could revolutionize how pancreatic cancer is treated by customizing interventions based on the patient's makeup. The journey towards applying these findings in world settings necessitates more investigation focused specifically on improving accuracy in targeting certain areas of treatment delivery and ensuring patient safety is paramount, as well as evolving the CRISPR technology responsibly by weighing its healing capabilities against ethical concerns to ensure progress benefits patients fairly and morally.

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