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CRISPR/Cas technologies in pancreatic cancer research and therapeutics: recent advances and future outlook

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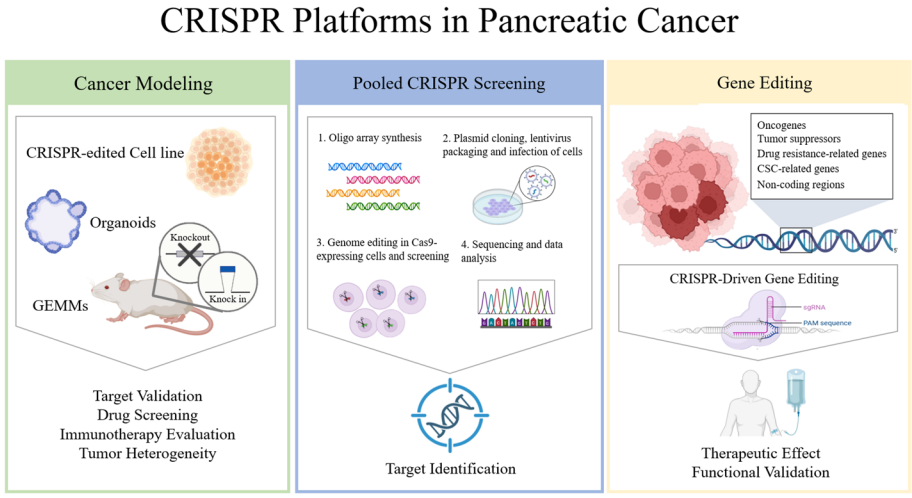
Abstract

Pancreatic cancer is marked by a poor prognosis and an exceptionally high mortality rate, with its aggressive nature contributing to its classification as a highly malignant disease. For effective therapeutic strategies, the development of sophisticated and regulated DNA manipulation methods is essential. Originally part of the prokaryotic immune system, CRISPR/Cas has emerged as a pivotal genome-editing tool with promising applications in pancreatic cancer research and therapy. This gene editing method is known for simplicity, rapid advancement, and superior precision compared to earlier techniques. Its adaptability allows precise gene editing for therapeutic purposes, including oncogene silencing and correction of pathogenic mutations. Additionally, CRISPR-driven gene editing has facilitated the development of pancreatic cancer models, which serve as valuable platforms for drug discovery and personalized treatment strategies, offering deeper insights into the genetic landscape of pancreatic tumors. This article provides an overview of the current applications of CRISPR technology in gene therapy and cancer research, particularly in the context of pancreatic cancer, and lays the foundation for future studies.

Keywords CRISPR, Pancreatic cancer, Genome editing, Gene-editing, Pancreatic cancer models

Graphical abstract



1 Background

Pancreatic ductal adenocarcinoma (PDAC) is the dominant form of pancreatic cancer, representing over 90% of cases worldwide. It ranks as the seventh major cause of cancer-related mortality globally [1]. The pathogenesis of pancreatic cancer involves several key genes, including the oncogenes Kirsten rat sarcoma viral oncogene homolog (KRAS) and MYC, as well as tumor suppressor genes such as Tumor Protein p53 (TP53), SMAD Family Member 4 (SMAD4), and Cyclin Dependent Kinase Inhibitor 2 A (CDKN2A) [2]. The disrupted signaling pathways implicated in pancreatic tumorigenesis include the Epidermal Growth Factor Receptor (EGFR), Notch, Hedgehog, and Wingless-related integration site (Wnt) pathways [3]. Besides, epigenetic modifications, including DNA methylation and histone modification, are also contributing factors in the aggressive behavior and therapeutic resistance of the tumor [4]. Resistance to chemotherapy often arises due to genetic mutations that favor the expression of drug resistance genes, thus leading to treatment failure [5]. All these molecular mechanisms need to be understood for further management and development of better therapeutic strategies.

Current treatments for pancreatic cancer, including surgery, chemotherapy, radiotherapy, and emerging immunotherapies, face significant limitations. Surgical resection offers the only chance for a cure but is feasible for few patients due to late diagnosis and frequent recurrence. Chemotherapy provides modest survival benefits but is often accompanied by severe side effects, while radiotherapy's effectiveness is hindered by tumor radioresistance and potential harm to surrounding tissues [6]. Immunotherapy, which includes immune checkpoint inhibitors and CAR-T cell therapy, has shown potential in the treatment of pancreatic cancer; however, the highly immunosuppressive tumor microenvironment that is characteristic of this malignancy presents a major obstacle to effective immuno-oncological approaches. Targeted therapies are developed to target distinct genetic mutations in cancer cells. Despite some success, the heterogeneity of pancreatic tumors and the development of resistance still pose a major challenge [7].

Modern biotechnology has advanced precise and targeted genetic manipulation tools, including Zinc Finger Nucleases (ZFN), Transcription Activator-Like Effector Nucleases

(TALENs), and the widely adopted CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats) systems, which have revolutionized genomic research and applications. Given the therapeutic potential of CRISPR/Cas technologies in solid tumors and the urgent need for innovative solutions in managing pancreatic cancer, this review explores the current applications, challenges, and future directions of CRISPR-based strategies in both research and treatment of this devastating malignancy.

2 The principle of the CRISPR/Cas system

The CRISPR/Cas system is a revolutionary genome-editing tool that has transformed biomedical research and therapeutic development by enabling targeted and precise genetic modifications [8]. Originally discovered as a bacterial adaptive immune mechanism, the system captures short sequences from invading viruses and plasmids to form a genetic memory of past infections. In bacteria, this immune system is organized into CRISPR arrays made up of alternating repeat and spacer sequences, which serve as templates for the production of guide RNAs [9, 10]. The mechanism of action involves RNA-guided nucleases that seek out and cleave specific nucleic acid sequences, a principle now widely applied for genome editing across various organisms [11, 12]. To simplify the process, researchers have engineered a single-guide RNA (sgRNA) by fusing the crRNA and tracrRNA components, thereby streamlining gene targeting and editing experiments [13].

A critical feature for nuclease activity is the presence of a protospacer adjacent motif (PAM) immediately downstream of the target sequence, ensuring specificity in recognition and cleavage [14]. For example, the popular Cas9 enzyme from Streptococcus pyogenes requires an NGG PAM sequence, a constraint that has been leveraged to attain high specificity in genome targeting [15]. Once the Cas protein binds to its target, it introduces a DSB in the DNA, which then activates the cell's own repair mechanisms [16]. These repairs primarily follow two pathways: homology-directed recombination (HDR) and non-homologous end joining (NHEJ), each producing distinct outcomes in the context of genome editing [16]. The error-prone NHEJ pathway often introduces small insertions or deletions (indels), thereby disrupting gene function and serving as an effective method for generating gene knockouts. In contrast, HDR facilitates precise genetic modifications when a donor template is supplied, although its application is largely confined to dividing cells. This error-prone nature of NHEJ has been effectively exploited to inactivate genes for functional genomics studies and disease modeling, while HDR-based strategies offer therapeutic avenues for correcting pathogenic mutations or inserting exogenous sequences [16, 17].

dCas9 (dead Cas9) is a catalytically inactive variant of the CRISPR-associated protein Cas9, engineered through point mutations (D10A and H840A) that abolish its endonuclease activity while retaining DNA-binding capabilities, enabling its use for precise transcriptional regulation without altering the genome [18, 19]. CRISPR interference (CRISPRi) employs dCas9 fused to repressive domains like Krüppel-associated box (KRAB) to block transcription initiation or elongation by sterically hindering RNA polymerase, achieving up to 99.9% gene repression in bacteria and 90% in eukaryotes [20, 21]. Conversely, CRISPR activation (CRISPRa) utilizes dCas9 fused to transcriptional activators (e.g., VP64 or p65AD) to recruit RNA polymerase and enhance gene expression, often targeting promoter regions near transcriptional start sites [22, 23]. Both

systems rely on sgRNAs for sequence-specific targeting, offering advantages over RNA interference (RNAi) by acting at the DNA level, avoiding competition with endogenous RNA machinery, and enabling modulation of noncoding RNAs and promoter regions [24, 25].

Beyond the conventional CRISPR/Cas9 system, several Cas protein variants, such as Cas12 and Cas13, have been identified, each possessing unique properties and expanding the technological applications. Cas12, for instance, operates with a different PAM requirement and produces staggered DNA cuts that can improve editing outcomes under certain conditions [26]. Meanwhile, Cas13 targets RNA instead of DNA, providing a powerful means for transcriptome engineering and modulating gene expression without permanently altering the genome [27, 28]. Owing to its modularity and programmability, CRISPR is widely adopted as a cost-effective and flexible alternative to earlier genome-editing tools such as ZFNs and TALENs [29, 30].

Despite these many advantages, the CRISPR/Cas system is not without its challenges, most notably the risk of off-target effects where the Cas nuclease may cleave unintended genomic sites [31]. To mitigate this, researchers have developed high-fidelity Cas variants and refined sgRNA design strategies to enhance precision and reduce off-target activity. Additionally, improvements in delivery systems, including the use of viral vectors and nanoparticles, have increased the efficiency of transporting CRISPR components into target cells for in vivo applications, while transient delivery methods such as ribonucleoprotein (RNP) complexes further limit off-target risks by reducing the duration of Cas nuclease activity [32–34].

Emerging innovations such as base editors and prime editors represent significant strides in precision genome editing. Base editors enable the direct conversion of one nucleotide to another without inducing DSBs, thus minimizing unintended indels [35]. Prime editors combine a Cas9 nickase with an engineered reverse transcriptase and a prime-editing guide RNA (pegRNA) to facilitate versatile and precise genome modifications, including insertions, deletions, and point mutations, which further expands the therapeutic potential of CRISPR applications [36, 37].

Overall, the CRISPR/Cas system's simplicity, efficiency, and versatility have not only revolutionized genetic engineering but have also paved the way for innovative research methodologies and therapeutic strategies, fundamentally transforming the landscape of genome editing [38].

3 Application of CRISPR/Cas systems in pancreatic cancer research

3.1 CRISPR-based pancreatic cancer modeling

In the field of cancer research, the generation of genetically engineered PDAC models is continuously needed to elucidate the molecular causes of PDAC development and validate the biological significance of newly identified therapeutic targets [39]. Additionally, in the drug development pipeline, highly predictive preclinical models of pancreatic cancer are essential to mitigate the costs of drug development and the likelihood of a failed clinical trial [40]. In order to produce precise animal models of cancer, the tumor and its microenvironment must resemble the human disease in terms of genetics, physiology, and anatomy. The laboratory mouse, due to its small size, short reproductive cycle, and genetic resemblance to humans, is the most commonly used animal model in cancer research. Insertional mutagenesis and gene-targeting, as conventional methods for

generating genetically engineered mouse models (GEMMs), are exceedingly time-consuming and labor-intensive; however, gene editing techniques are recognized for their potential for simplifying this process [41]. It is feasible to generate GEMMs by manipulating embryonic stem cells and germ cells to produce homozygous mutant mice after one generation. Presently, alongside murine embryonic stem and germ cell manipulation, numerous genes are concurrently modified, indicating the progressive advancement of the CRISPR-based genome editing approach as a genetics toolkit in murine somatic cell editing [42].

The majority of PDAC GEMMs are driven by oncogenic KRAS and can replicate the molecular and histological characteristics of human PDAC and similar precursor lesions [43]. While KRAS mutations are necessary for the development of pancreatic disease, multiple mutations into a relevant tumor suppressor gene have been reported. Hence, knockout studies have been performed in the mutant KRAS context to elucidate the influence of these additional mutations on pancreatic tumorigenesis [44]. Haploinsufficiency of the tumor suppressor liver kinase B1 (Lkb1) has been reported to synergize with KRAS mutations, thereby accelerating pancreatic cancer progression [45]. Chiou et al. utilized a loxP-Stop-loxP (LSL) $KRAS^{G12D}$ knock-in mouse that integrates with a CRISPR/Cas9-mediated somatic genome editing strategy to create an in vivo pancreatic cancer model. In these models, Cas9 will only be expressed following stop cassette recombination triggered by transgenic Cre recombinase expression. Their findings indicated that CRISPR-mediated targeting of Lkb1 in the presence of oncogenic $KRAS^{G12D}$ resulted in accelerated tumor development [46].

Additionally, researchers have generated GEMMs with various histological subtypes for the generation of different pancreatic lesions [44]. Analogous to the previous study, Ideno et al. delivered multiplexed sgRNAs and a mutant template sequence to the p48-Cre; LSL-Cas9 mice using adeno-associated viruses (AAVs). This CRISPR-mediated gene editing system introduced an oncogenic $KRAS^{G12D}$ allele via HDR and knocked out the Trp53, Lkb1, and Arid1A. This genetic manipulation resulted in a range of precursor lesions that ultimately progressed to PDAC, leading to the development of PDAC GEMMs with complex genotypes [47]. Therefore, recent advancements in the CRISPR/Cas9 system have significantly enhanced the HDR efficacy and the introduction of gain-of-function mutations or allele replacements [48].

The toxic response triggered by Cas9-induced DSBs is dependent on functional p53, leading to reduced efficiency of precise genome editing in cells with an intact p53 pathway [49]. This p53-mediated DNA damage response represents a significant barrier to the effective application of CRISPR/Cas9 for genome engineering, complicating editing outcomes.

While GEMMs effectively replicate several key features of human PDAC, such as stromal desmoplasia, metastatic potential, and genomic instability, they fall short in capturing other important aspects of the disease. A key limitation lies in the reliance on tissue-specific promoters, which typically drive gene expression broadly during embryonic pancreas development. This often leads to the emergence of multifocal tumors with short latency periods, a pattern that does not accurately reflect the sporadic and delayed progression observed in human PDAC. Furthermore, engineering multiple genetic alterations in GEMMs is technically demanding and necessitates large animal cohorts to achieve statistically meaningful results. These constraints have hindered comprehensive

studies on the combined effects of co-occurring genetic alterations in tumor development [50]. To overcome these limitations, Maresch et al. introduced an innovative CRISPR/Cas9-based strategy to selectively induce defined genetic lesions directly in the adult pancreas in vivo, enabling more precise modeling of PDAC-associated mutations.

Generally, electroporation has been known as a rather inefficient transfection method, only transfecting a limited number of cells. However, Maresch et al. conducted an in vivo multiplex mutagenesis study demonstrating that electroporation facilitates the transfection of multiple plasmids and enables the delivery of the CRISPR/Cas9 system to the pancreas of Ptf1-Cre; LSL $KRAS^{G12D}$ mice, thereby allowing simultaneous editing of several gene sets. Using the CRISPR/Cas9 system, this study successfully indicates the susceptibility of pancreatic cells to BRCA2 inactivation in KRAS-mutant cells [51]. Although the study included a highly efficient guide RNA targeting BRCA2, no BRCA2-edited alleles were detected in the resulting tumors. This absence suggests that certain genes, such as BRCA2, may either be subject to strong negative selection during tumor development or may resist editing altogether under the given conditions. Interestingly, the in vivo CRISPR/Cas9 screening technique that is mentioned in the following could facilitate the establishment of a new animal model related to pancreatic cancer, as well as the discovery of novel gene functions [52].

Recently, a pancreatic cancer GEMM was also developed through the temporal regulation of base editor activity utilizing inducible base editing (iBE) capabilities. This model employs a transgenic cytosine base editor (CBE) regulated by a tetracycline response element, facilitating the efficient modification of single or multiple single-nucleotide variants in pancreatic organoids. In addition, direct delivery of sgRNAs to target tissues in the iBE system makes it easier to develop in situ preclinical cancer models [53]. To facilitate functional studies of complex genetic combinations that are difficult to construct using traditional models, in vivo prime editing mouse models were also created by encoding a Cre-inducible prime editor in the mouse germline. This strategy engineered a variety of mutations, including KRAS and Trp53 mutations frequently observed in pancreatic cancer, through in vivo somatic prime editing [54].

While GEMMs can be helpful in assessing the immunotherapy efficacy due to the existence of a complete immune system [44], their ability to accurately predict the PDAC therapeutic response is hampered by species-related variations between human tumors and GEMMs. In fact, the degree of aneuploidy in human tumors results in significant intertumoral gene alterations; thus, utilizing transplantation models can mitigate these limitations [55]. Additionally, the anatomical differences between murine models and humans hinder the formation of tumors that are analogous in size and location to those in humans. Hence, the utilization of PDX models in bigger animals is increasingly promising. In 2021, Hendricks-Wenger et al. employed RAG2/IL2RG-deficient pigs as an innovative model to investigate pancreatic cancer. In this study, the RAG2 and IL2RG genes are disrupted in fertilized oocytes using the CRISPR/Cas9 system to impair the pig immune system. Then, xenograft tumors were cultivated from human Panc01 cells in immunocompromised pigs. This successful engraftment exhibited growth rates comparable to those generally observed in mouse models, with no indications of host immune rejection [56]. It is worth noting that the CRISPR/Cas9 gene editing toolkit additionally eliminates transplant rejection by the host immune system without requiring immunocompromised animals, thus providing a foundation for novel PDAC models [57].

In addition to creating precise mutations involving single nucleotides or smaller genomic segments, as previously mentioned, CRISPR technology also enables the generation of substantial chromosomal aberrations. The CRISPR-mediated rearrangement (CRISMERE) strategy has demonstrated significant efficacy in generating desired alterations through the creation of substantial deletions, duplications, and inversions [48, 58], and it is expected to be employed in pancreatic cancer research in the near future. Wild-type Cas9 and the nickase mutant Cas9 variant have also been predominantly utilized in the development of mouse cancer models. Although dCas9 does not induce genome modifications, its impact on the expression of target genes is revealed in in vivo cancer models [59].

Primary pancreatic tumor cell lines serve as accessible ex vivo cellular models for pancreatic cancer, offering significant practical advantages, such as the feasibility of high-throughput screening to identify genetic targets, effective chemical compounds, or potential biomarkers in a cost-effective and simple manner [60]. However, the translation of these studies into clinical practice is challenging [61]. Utilizing the CRISPR/Cas9 methodology, Monazzam et al. successfully established stable MEN1 knockout BON1 cells as a model for pancreatic neuroendocrine tumors (PNET) with mutations in the MEN1 tumor suppressor. In the following, the morphological, proliferation, and proteomic analyses demonstrated enhanced proliferation, changed protein profiles, and altered biological functions associated with cancer [62]. The CRISPR system is additionally advantageous for enhancing existing cancer models by reengineering embryonic stem cell lines derived from established transgenic mice to create more intricate mutation alleles of oncogenes and tumor suppressor genes [48].

Alongside conventional monolayer cell lines, GEMMs, and PDXs, three-dimensional ex vivo cultures known as organoids have recently garnered significant interest in pancreatic cancer research [63]. In fact, this three-dimensional and self-organizing cultured system combines two methods for evaluating drug sensitivity: xenotransplantation of the tumor into immunocompromised mice and short-term culture of tumor sections. To validate the in vitro assessment, patient-derived organoids also can be used in the PDX context [64]. Additionally, these organoids can be derived from minimal biopsies of pancreatic cancer tissues, facilitating the examination of localized, advanced, and metastatic cases [61].

Although most cancer research studies utilize adult stem cell-derived organoids from primary cancer samples, CRISPR technology has modified pluripotent stem cell-based organoids to produce cancer-causing mutations [52]. To model pancreatic cancer genetically, numerous studies employed CRISPR-mediated genome editing to modify driver genes in pancreatic cancer organoids [65, 66]. Lee et al. induce permanent mutations in KRAS, CDKN2A, SMAD4, and TP53 in primary human duct cells through lentiviral delivery combined with the CRISPR/Cas9 system, resulting in the creation of engineered pancreas organoids. In this study, KRAS overexpression leads to pancreatic intraepithelial neoplasia (PanIN) modeling [65]; however, the KRAS knock-in in Seino et al.'s research resulted in the creation of organoid-derived tumors that were histologically similar to PDAC. Using sequential CRISPR-mediated editing, Seino and associates produced a significant cohort of PDAC organoids derived from patients. This editing tool disrupts the CDKN2A, TP53, and SMAD4 genes and introduces the $KRAS^{G12V}$ mutation to illustrate tumor heterogeneity [66].

Patient-derived organoids that replicate pathological states even offer an excellent platform for screening and evaluating therapeutic agents. In this process, surgical specimens can be utilized to establish an organoid biobank to optimize treatment options [67]. Recently, Hirt and associates developed a biobank of 31 patient-derived organoids that reflected the PDAC heterogeneity. Through the implementation of CRISPR/Cas9 genome modification and drug screening, ARID1A was found to be associated with heightened sensitivity to dasatinib and VE-821 [68]. Another study used the isogenic murine pancreatic organoids with multi-hit oncogenic mutations introduced by CRISPR/Cas9 technology for high-throughput drug screening. This screening identified perhexiline maleate as a growth inhibitor and apoptosis inducer in PDAC organoids [69].

CRISPR systems, particularly those utilizing Cas9, have emerged as powerful tools for modeling pancreatic cancer by enabling the generation of organoids, GEMMs, and genetically engineered cell lines that reflect the complex genomic landscape of human tumors. Despite their promise, CRISPR-based organoid models present notable limitations. One key challenge is the variability in growth rates among organoids, which can distort the outcomes of pooled CRISPR screens. This distortion arises when faster-growing clones dominate the culture, artificially enriching the associated gRNAs and potentially skewing the interpretation of results [70]. The lack of tumor microenvironment elements also diminishes the physiological relevance of organoid models, while inconsistencies in culture conditions and assay methodologies hinder reproducibility and limit the comparability of findings across studies [71].

Another limitation, not necessarily confined to organoid systems, involves the simultaneous introduction of multiple gRNAs into a single cell. This approach can confound data interpretation by creating complex genomic alterations that are difficult to attribute to specific guides. However, as the methodology matures and standardized protocols are established, this challenge is expected to diminish progressively [70]. For instance, Liang et al. addressed this issue by excluding potentially misleading data, removing all cells containing both functional and passenger guide RNAs from downstream analyses [72].

GEMMs are important preclinical platforms that enable the in vivo study of complex disease processes and complement other experimental models. Despite their utility, several critical questions remain open when utilizing these models. For example, it is essential to investigate whether tumors with varying histopathological characteristics exhibit differences in the frequency of target gene alleles or whether they accumulate additional mutations over time. Moreover, evaluating how the overall mutation burden and neoantigen landscape in GEMMs compare with those in human tumors can help clarify their clinical relevance. The tumor microenvironment, especially the stromal response, which is particularly intense in cancers like pancreatic ductal adenocarcinoma, also requires careful examination to determine how closely GEMMs reflect human stromal features. Finally, these models offer a unique opportunity to link mutational profiles with treatment responses, an essential component in the advancement of novel therapeutic strategies in translational research.

Figure 1 depicts the methodologies employed for establishing pancreatic cancer models utilizing various CRISPR-based systems.

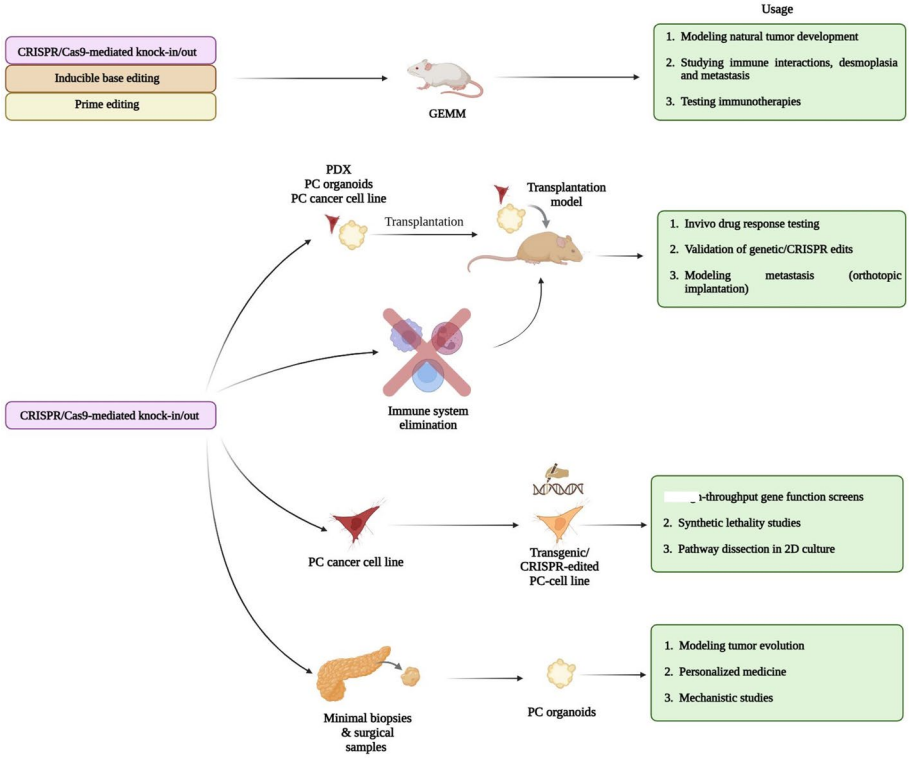


Fig. 1 Generation of pancreatic cancer models employing a range of CRISPR systems. Various CRISPR gene editing techniques are instrumental in generating GEMMs. Among them, CRISPR/Cas9 plays a pivotal role in the creation of transplantation models, either by modifying the genome of pancreatic cancer cells or by manipulating the immune system to facilitate PDX models. This system has also been widely applied in generating transgenic pancreatic cancer cell lines and genetically modified organoids, advancing research in cancer biology and therapeutic development

3.2 New target discovery by crispr/cas screening

CRISPR screening has emerged as a key method for uncovering gene functions, revealing molecular mechanisms across basic biology, medical genetics, and cancer research [73]. Primary CRISPR screening strategies share a common workflow and include gain-of-function approaches like CRISPRa, as well as loss-of-function methods such as CRISPR knockout and CRISPRi. In a standard pooled CRISPR screening, a plasmid library containing thousands of sgRNAs, each targeting a specific gene, is introduced into cell pools expressing the appropriate Cas9 variant via lentivirus or retrovirus transduction. The selection of the Cas9 variant is dependent on the screening objective: Cas9 is utilized for CRISPR knockout, dCas9 paired with a transcriptional repressor is applied for CRISPRi, and dCas9 paired with a transcriptional activator is employed for CRISPRa. The transduced cells are subsequently subjected to selection processes and cultured under defined conditions to isolate cells with a desired phenotype [74]. Afterward, genomic DNA is extracted from the selected cells, and next-generation sequencing (NGS) along with bioinformatics software is employed to measure sgRNA differences between controls and treated cells [75]. Figure 2 illustrates these steps in addition to the diverse CRISPR screening strategies conducted in in vivo and in vitro platforms.

In the cancer research area, CRISPR screening is utilized for gene functions across tumor development, metastasis, therapeutic resistance, and immunotherapy efficacy. These high-throughput analyses are conducted either in vitro or in xenograft models

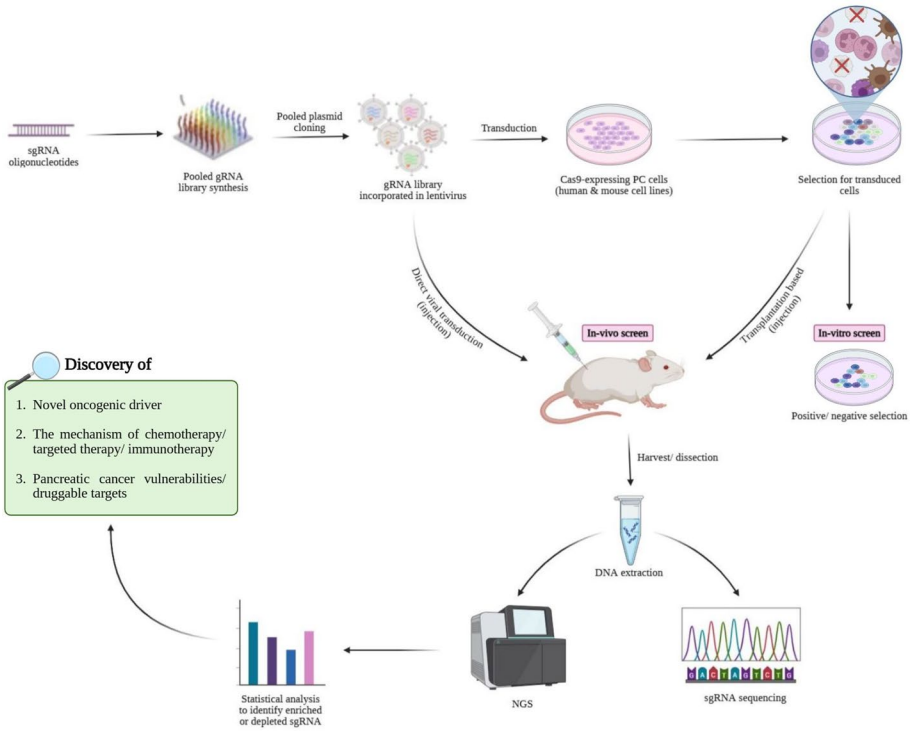


Fig. 2 Pooled CRISPR screening approaches in different experimental conditions. In direct in vivo screening, CRISPR is delivered into living organisms (e.g., mice) to induce genetic modifications in their natural biological context. In indirect in vivo screening, CRISPR is applied to cell lines or organoids derived from the in vivo model, which are then reintroduced into the organism, allowing for controlled exploration of genetic modifications. In vitro CRISPR screening is conducted in cultured cells for high-throughput gene editing and analysis of specific genetic targets. Sequencing technologies, such as NGS, are then used to identify novel oncogenes and druggable targets, providing insights into gene functions and the impact of specific genetic changes in isolated cells

[76]. Additionally, in recent years, researchers have employed direct in vivo CRISPR screens to identify the oncogenic drivers of tumorigenesis within the native pancreatic cancer microenvironment [77]. Table 1 presents CRISPR screening experiments in detail.

CRISPR knockout screening has enabled the identification of novel oncogenic drivers specifically involved in metastasis [75, 96]. In this setting, Bakke et al. perform a CRISPR screening to identify novel essential genes in PDAC by transfecting a human CRISPR Brunello knockout library into Cas9-expressing PANC-1 cells. Following the analysis of NGS results, the proteasome subunit alpha type 6 (PSMA6) gene was identified as the top hit and validated through siRNA screens. The findings indicate that PSMA6, which encodes a proteasome subunit, may serve as valuable druggable candidates for PDAC [78]. The CRISPR/Cas9 screening conducted by Oladimeji et al. with the same library and similar cell line. According to findings, Muscleblind Like Splicing Regulator 3 (MBNL3) and KAT8 Regulatory NSL Complex Subunit 2 (KANSL2) significantly regulate invasiveness in pancreatic cancer cells, which were revalidated using both CRISPR knockout and siRNA knockdown techniques [79]. In 2024, Martinez et al. concentrate on the utility of in vivo CRISPR/Cas9 screens for the identification of long-tail PDAC genes and their associated pathways that collaborate with oncogenic $KRAS^{G12D}$ in cancer development. In this study, Ubiquitin Specific Peptidase 15 (USP15) and SR-Related CTD Associated Factor 1 (SCAF1) have been identified as pancreatic tumor suppressors

Table 1 Recent studies on CRISPR screening application in pancreatic cancer

Selection condition	Model type	CRISPR screening type	CRISPR library	Top hits	Validation technique	Effect	Ref.
100 nM gemcitabine for 6 days	PANC-1	Knockout	Brunello CRISPR library (addgene No. 73178)	PSMA6	siRNA screens	-Enhanced cellular apoptosis -Reduced spheroid formation	[78]
2 µg/mL of puromycin for 8 days then absence of puromycin for 5 days	PANC-1	Knockout	Brunello CRISPR library (addgene No. 73178)	MBNL3, KANSL2	CRISPR knockout, siRNA knockdown	-Impaired invasiveness	[79]
–	Pdx1-Cre; LSL-KrasG12D; LSL-Cas9-GFP mice	Knockout	Pooled library from sgRNAs targeting long tail genes [80] and non-targeting sgRNAs [81] not include sgRNAs targeting Trp53 or Smad4	USP15, SCAF1	CRISPR knockout	-Increased proliferation -Shorter disease-free survival -Increased sensitivity to Olaparib in vitro and in vivo	[77]
10% FBS and 10% LPDS conditions after 12 days, nude mice injection	Pa03c In vitro and in xenograft model	Knockout	SREBP target sgRNA library	SREBPs	CRISPRi knockdown	-Reduced tumor burden in an orthotopic xenograft mouse model	[82]
10 µg/mL puromycin for 3 days	TB32047	Knockout	GeCKOv2 library (addgeneNo. 1000000053)	DCK, CCNL1	CRISPR knockout	-Enhances Resistance to Gemcitabine -Activated the ERK/AKT/STAT3 pathway	[83]
25 nM Gemcitabine, 2.5 µm Oxaliplatin, 500 nM Irinotecan, and 7.5 µm 5-FU (for PANC-1) and 25 nM Gemcitabine, 2.5 µm Oxaliplatin, 250 nM irinotecan, and 5 µm 5-FU (for BxPC3) for 4 days	Panc-1 and BxPC3	Knockout, Activation	GeCKO library (addgeneNo.1000000049), SAM pooled sgRNA library (addgene No. 1000000057)	HDAC1, VPS25, ABCG2	CRISPRa, CRISPR knockout	-Increased expression of several genes involved in the EMT (Following increased HDAC1)	[84]
10 µg/mL puromycin for 3 days	TB32047	Knockout	The pooled sgRNA library (Mouse Brie kinome pooled library) (addgene No. 75316) with additional sgRNAs for genes involved in pancreatic cancer	CDK7	CRISPR knockout, Pharmacological inhibition	-Induced cell cycle arrest Increased apoptosis and DNA damage -Increased gemcitabine and paclitaxel sensitivity -Reduced tumor growth in vivo	[85]
Puromycin for 7 days	Pa-Tu-8988T and YAPC	Knockout	GeCKOv2 library	PKMYT1	CRISPR knockout, Pharmacological inhibition	-Increased apoptosis -Induced cell cycle arrest	[86]
100 nM trametinib for 14 days, 10 nM trametinib for 14 days	PATU8902, PATU8988T	Knockout	GeCKOv2 library (addgene No. 1000000049) and Avana library [87]	CIC, ATXN1L	CRISPR knockout	-Reduced sensitivity to trametinib	[88]

Table 1 (continued)

Selection condition	Model type	CRISPR screening type	CRISPR library	Top hits	Validation technique	Effect	Ref.
2 µg/ml of puromycin for 3 days	PANC-1	Knockout, Suppression	Brunello CRISPR library (addgene No. 73178), CRISPR Dolcetto library (addgene No. 92386)	BUB1B, BUB3, and TTK	shRNA knockdown, Pharmacological inhibition	-Decreased nab-paclitaxel sensitivity -Decreased proliferation (Inconsistency with pharmacological inhibition outcomes)	[89]
2 µg/ml puromycin at different time point (Day 15, 27, 31, 35)	HPAF-II, AsPC-1, PaTu8988S	Knockout	Toronto KnockOut (TKO) CRISPR Library (addgene No. 1000000069)	Wnt pathway genes, FZD5	CRISPR knockout, Antibody-mediated inhibition	-Reduced proliferation	[90]
MEK and CENPE inhibitor treatment in 6–8-week-old male athymic nude mice	PDX366 (In vitro and In vivo)	Knockout	Nuclear proteins gRNA sub-pool library (addgene No. 51047)	CENPE, RRM1	CRISPR knockout, Pharmacological inhibition	-Increased sensitivity to trametinib	[91]
1.5 µg/mL puromycin for 9–13 days	HupT3, KP4_MSLN	Knockout	Brunello lentiviral library (Genetics Perturbation Platform, Broad Institute)	TFAP4	CRISPR knockout	-Altered the activity of the NFκB transcription factor p65	[92]
1.5 µg/ml puromycin for 5 days	KPC3-OVA cells	Knockout	Brie CRISPR Knockout Library (addgene No. 73632)	EGFR, MFGE8	CRISPR knockout	-Sensitized mesenchymal-like cancer cells to CTL-mediated killing	[93]
Puromycin	KPC^{mut} cells In vitro and In vivo	Knockout	sgRNA library targeting the kinome and membranome	RIPK2	CRISPR knockout, shRNA knockdown, Pharmacologic inhibition	-Improved efficacy of anti-PD-1 immunotherapy	[94]
–	KPCY mouse model-derived cell lines	Knockout	Constructed sgRNA library targeting epigenetic and RNA-binding factors	KDM3A	CRISPR knockout	-Increased immune infiltration -Increased sensitivity to immunotherapy	[95]

that modulate inflammatory responses and sensitivity to Olaparib [77]. The significance of the sterol regulatory element-binding proteins (SREBPs) and the terpenoid backbone biosynthesis pathway for PDAC growth was also demonstrated by in vivo CRISPR screening, indicating potential for treatment by focusing on lipid metabolism [82].

Genome-wide CRISPR screening facilitates understanding molecular mechanisms of drug response by introducing sgRNA libraries into cancer cells [97, 98]. Chemoresistance significantly limits the efficacy of chemotherapeutic agents such as gemcitabine in pancreatic cancer treatment. Accordingly, most studies on chemoresistance have focused on gemcitabine. Recently, Yang et al. employed a genome-scale CRISPR knockout GeCKO library to identify loss-of-function mutations that confer gemcitabine resistance in the TB32047 mouse pancreatic cancer cell line. They revealed that the elimination of Deoxycytidine Kinase (DCK) and cyclin L1 (CCNL1) enhances resistance to gemcitabine treatment [83]. To evaluate cellular resistance mechanisms to chemotherapeutic agents, Ramaker et al., in another study, employed genome-wide CRISPRa and CRISPR knockout screens utilizing the SAM and GeCKO pooled sgRNA library transfection in PDAC cells, respectively. In this study, CRISPRa of the ATP-binding cassette (ABC) transporter (ABCG2) is the most reliable factor of resistance across all screens. CRISPR-mediated activation of genes involved in transcriptional co-repressor complexes additionally produced multidrug resistance. The upregulation of these genes, including Histone Deacetylase 1 (HDAC1), enhances promoter occupancy and induces the expression of multiple genes involved in epithelial-to-mesenchymal transition (EMT) and migratory phenotypic changes. However, inhibition of Vacuolar Protein Sorting 25 Homolog (VPS25), which emerged as one of the top candidates in the CRISPR knockout screen, produced inconsistent results [84]. In another study, a genome-wide CRISPR/Cas9 loss-of-function screening identified cyclin-dependent kinase 7 (CDK7) as a top hit among various cell cycle checkpoint and DNA damage-related kinases for addressing chemoresistance [85].

A recent study indicates that PKMYT1 was identified as a genetic susceptibility and a therapeutic target for PDAC via whole-genome CRISPR/Cas9 loss-of-function screens [86]. This method in another study also demonstrated that the knockout of genes within the PI3K/AKT/mTOR pathway improved the effectiveness of CDK4/6 inhibitors in pancreatic cancer [99]. For the further discovery of genes that influence sensitivity to MEK and RAF inhibitors (MAPKi), Wang et al. also conducted CRISPR/Cas9 knockout screening by transfecting GeCKOv2 and Avana libraries into the KRAS mutant pancreatic cancer cell lines PATU8902 and PATU8988T, respectively. The comparative analysis results indicate that both CIC and ataxin-1-like (ATXN1L) serve as mediators of resistance to MEK1/2 inhibitor treatment [88]. A recent genome-wide CRISPR interference and knockout screening study identified a number of spindle assembly checkpoint (SAC) genes, including BUB1B, BUB3, and TTK, which improve the survival of nab-paclitaxel-treated cells. These SAC genes are essential for proper segregation of chromosomes throughout cell division, and their knockdown reduced cytotoxic effects of nab-paclitaxel [89].

For precision medicine, gene editing is used to find and validate new biological targets, and CRISPR tools for functional genomic screening are becoming more and more crucial in achieving this aim [100]. To identify potential vulnerabilities in pancreatic cancer cells, Steinhart et al. performed a CRISPR/Cas9 screen. They transfected a TKO

gRNA library into RNF43-mutant PDAC cells expressing Cas9 and conducted screenings at different time points. The analyzed data revealed WLS, CTNNB1, TCF7L2, LRP5, and PORCN as key elements of the Wnt pathway, along with FZD5, WNT7B, and WNT10A as critical genes for HPAFII cell proliferation. This study identified the Wnt signaling circuit as an essential driver of pancreatic cancer cell growth [90]. Additionally, it is obvious that the ultimate objective of cancer research and precision medicine is to predict responses and identify additional targets based on individual characteristics that enhance chemotherapy efficacy [91]. Researchers have identified the RAS/RAF/MEK/ERK axis as the primary catalyst of tumorigenesis, providing survival signals to cancer cells [101]. In 2018 Based on a large-scale in vivo and in vitro CRISPR knockout screening, Szlachta et al. identified genes whose knockout regulates the survival of MEK-inhibited PDAC cells. They transfected a nuclear sgRNA library, developed by Wang et al. [102], targeting 3733 nuclear protein genes into a PDX model and identified depleted genes that synergistically enhance the cytotoxicity of trametinib as a MEK inhibitor. They selected CENPE and RRM1 among the top hits, validated them, and found that ablated or inhibited cells were significantly more sensitive to trametinib treatment [91].

The advantages of CRISPR-based screens extend beyond identifying genes associated with tumorigenesis or chemoresistance; they also encompass the investigation of genetic changes that provide resistance to immunotherapeutic strategies and immune evasion. In 2023, mesothelin-expressing pancreatic cancer cells were subjected to genome-scale CRISPR/Cas9 loss-of-function screens in order to assess resistance to CAR T-cell therapy. Infection of pancreatic cancer cells with the Brunello lentiviral library, followed by puromycin selection and co-culture with mesothelin-specific CAR T cells, revealed that disruption of GPI-anchor biosynthesis genes impaired the ability of CAR T cells to suppress tumor growth. Furthermore, TFAP4 knockout results in an elevation of the NFκB transcription factor p65, suggesting that CAR T-cell therapy resistance likely entails modifications in intrinsic tumor states [92].

Additionally, Gu et al. employed genome-wide CRISPR/Cas9 knockout screens in both epithelial and mesenchymal-like pancreatic cancer cells, and EGFR and MFGE8 were identified and validated as Mes-specific regulators of cytotoxic T lymphocyte (CTL)-mediated killing. Elevated expression of EGFR and MFGE8 in pancreatic cancer cells was associated with a poorer prognosis, promoting immune evasion from CD8 + T cells, and their knockout rendered mesenchymal-like cancer cells more susceptible to CTL-mediated cytotoxicity [93]. In a 2024 study, through in vivo CRISPR screening in orthotopic PDAC models, Sang et al. identified receptor-interacting protein kinase 2 (RIPK2) as a pivotal mediator in the immunosuppressive TME by inhibiting antigen presentation and cytotoxic T-cell activity [94]. This in vivo approach also validated an epigenetic regulator, lysine demethylase 3 A (KDM3A), which influences the immune environment in PDAC, indicating that its knockout enhances immune infiltration and sensitivity to immunotherapy [95].

These studies effectively demonstrate that CRISPR pooled library screening under specific selection conditions can reveal novel gene functions in the context of pancreatic cancer. This approach offers a powerful strategy to identify therapeutic vulnerabilities in pancreatic cancer cells and to guide the development of new treatment options. However, several factors may influence the accuracy of screening results. The number of cells required for transduction varies depending on the size of the library to ensure adequate

coverage. While weak selection pressures can yield more comprehensive data, they also increase the risk of false positives that may not contribute to phenotypic changes. Additionally, different selection methods can introduce experimental variability. The quality of deep sequencing and the choice of data analysis tools are also critical for accurate screening outcomes. Therefore, it is essential to validate findings using orthogonal methods.

4 Applications of CRISPR/Cas systems as therapeutics in pancreatic cancer

The CRISPR/Cas system represents a transformative approach in oncology, offering the ability to precisely edit genetic alterations associated with tumorigenesis, thereby paving the way for the development of more targeted and efficacious cancer therapies [103]. Therapeutic applications of CRISPR-mediated gene editing investigated in pancreatic cancer are summarized in Fig. 3, and the following studies address various aspects of this issue.

4.1 Oncogenes editing

Intensive investigation is underway regarding the potential of CRISPR to target oncogenic mutations associated with cancer development [104], as well as the elimination of the mutant KRAS function in different cancer cells and animal models [105]. For this aim, CRISPR knockout entirely eliminates gene expression and could result in very

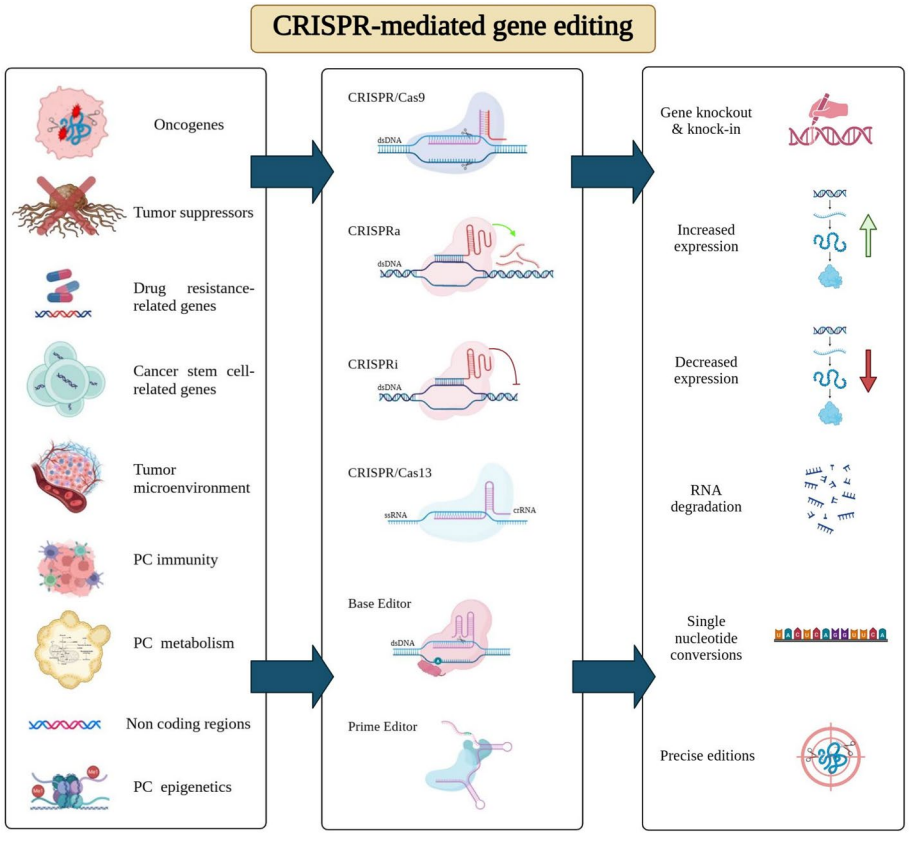


Fig. 3 Therapeutic applications of CRISPR-driven gene editing. Through the targeted modification of key factors involved in the pathogenesis of pancreatic cancer, CRISPR systems, employing diverse mechanisms, offer promising prospects for advancing therapeutic strategies in the treatment of pancreatic cancer. *PC* pancreatic cancer

few off-target effects in contrast to the partial knockdown typically attained with RNAi [102].

KRAS is a member of the G-protein family and a regulator of cellular survival and proliferation. The mutations in this protein result in the inactivation of its GTPase activity, thereby causing aberrant effector signaling and the onset of pancreatic cancer [106]. The oncogenic KRAS mutation, an initial occurrence in pancreatic cancer development, is implicated in over 90% of cases, contributing to their unfavorable prognosis and resistance to targeted therapies; thus, silencing KRAS contributes to the suppression of pancreatic cancer progression [107, 108]. Nevertheless, therapeutic strategies for addressing cancers harboring such mutations have focused on targeting the downstream protein effectors of KRAS, as no approved treatment has directly targeted the mutated KRAS oncogene [109]. Using CRISPR, Muzumdar et al. became the pioneering group to examine the resistance mechanisms underlying KRAS suppression and discover KRAS-mediated mechanisms in pancreatic cancer. Notably, mutant KRAS cannot be differentiated from the wild type by the sgRNAs because of the lack of distinct PAM sites [110]. Indeed, consistent with earlier studies, only KRAS-dependent cells exhibited susceptibility to KRAS knockdown, resulting in a marked reduction in cell viability [111]. Until today, the CRISPR/Cas9 system has been employed to target KRAS mutant alleles, such as G12V, G12D, G12S, and G13D, to regulate tumor proliferation [109, 112, 113]. In 2018, Lee et al. demonstrated that CRISPR/Cas9-mediated knockout of two specific single-nucleotide missense mutations at KRAS codon-12, including G12V and G12D substitutions, was associated with diminished viability of cancer cells. These substitutions are among the most frequently observed mutations in pancreatic cancer and are linked to unfavorable prognosis [109]. One year later, Lentsch et al. eradicate heterozygous $KRAS^{G12D}$ in human and mouse pancreatic cancer cell lines by CRISPR/Cas9 technique. In this study, the $KRAS^{G12D}$ protein was successfully knocked out [114]. Knocking out the mutant $KRAS^{G12D}$ oncogenic allele by CRISPR/Cas9 was even conducted with exosomal delivery approaches [115]. In order to mitigate the risks associated with prolonged expression of bacterial Cas9, this platform was designed to encapsulate CRISPR/Cas9 payload as plasmid DNA or in a more transient format, specifically mRNA and RNP [116]. To target the oncogenic $KRAS^{G12S}$ for the first time, Gao et al. utilized two CRISPR systems: the genome-editing CRISPR/Cas9 and the transcription-repressing dCas9-KRAB. In the latter system, dCas9 interacts with the target sequence, while the transcriptional repressor KRAB inhibits mRNA transcription. Both gene-depletion and transcription-suppressing strategies aimed at specifically targeting the mentioned mutation while preserving the wild-type KRAS allele resulted in the inhibition of cancer cell proliferation both in vitro and in vivo [113].

In addition to the CRISPR/Cas9 system, Cas13a has been characterized as a crRNA-guided RNA-targeting CRISPR effector capable of binding to and cleaving target RNA with a complementary sequence [117, 118]. Zhao et al. developed the CRISPR/Cas13a platform for the precise targeting of $KRAS^{G12D}$ and $KRAS^{G12C}$ transcripts in pancreatic cancer. This study demonstrates that the bacterial Cas13a protein and crRNA effectively reduce mutant KRAS mRNA expression, revealing a CRISPR/Cas13a system capable of achieving a knockdown efficiency of up to 94% without affecting wild-type KRAS mRNA levels [119]. CasRx, a promising alternative for post-transcriptional gene silencing, is a Cas13d ortholog fused with a nuclear localization sequence, demonstrating

superior efficiency in cleaving target sequences to downregulate the expression of both coding and noncoding RNAs more selectively and effectively [120]. Furthermore, CasRx exhibited minimal off-target effects in mammalian cells and is significantly smaller than Cas9 [121]. In the research conducted by Jiang et al., the CRISPR/CasRx system was shown to effectively and precisely silence the mutant $KRAS^{G12D}$ transcript with exceptional specificity in PDAC cells. Their findings indicated that, with guidance from a $KRAS^{G12D}$-specific gRNA, CasRx was able to successfully silence the expression of the mutant $KRAS^{G12D}$ in PDAC cells. This knockdown led to the suppression of the aberrant activation of downstream signaling pathways induced by mutant $KRAS^{G12D}$, resulting in reduced tumor growth and enhanced sensitivity of PDAC cells to gemcitabine. This study provides a proof-of-concept that the CRISPR/CasRx system can be an effective approach for targeting and silencing mutant $KRAS^{G12D}$ transcripts to mitigate the malignancy of PDAC [122].

Despite the promise of CRISPR in targeting oncogenes, several challenges remain in selectively targeting mutant KRAS. A major technical limitation is the lack of unique PAM sites near key point mutations, which prevents precise editing of the mutant allele without affecting the wild-type allele. This limitation hinders the development of precise gene-editing strategies targeting oncogenic KRAS variants and leads to non-selective inhibition of KRAS, which is a common feature of most current CRISPR-based approaches under evaluation for KRAS targeting [110]. This PAM-related challenge is not exclusive to Cas9, the most widely used CRISPR-associated nuclease. Different Cas enzymes, such as Cas12a (Cpf1), recognize distinct PAM sequences, which can partially alleviate PAM constraints depending on the genomic context [26]. However, the core issue remains sequence-dependent and is intrinsic to all CRISPR systems requiring PAM recognition. Emerging gene-editing technologies like base editors and prime editors, which are engineered from Cas9 or Cas12 variants, offer improved precision by enabling targeted nucleotide changes without inducing double-strand breaks. These approaches can circumvent some limitations imposed by PAM availability and reduce off-target effects [123, 124], providing promising alternatives for allele-specific editing of oncogenic mutations such as those in KRAS.

Although frequently mutated driver genes such as KRAS, TP53, CDKN2A, and SMAD4 are associated with a negative outcome in PDAC patients, they cannot currently be targeted by existing agents [125, 126], necessitating the identification of novel druggable targets. For example, TGF-β signaling plays a crucial role in PDAC tumorigenesis and exerts diverse effects in cancers through the SMAD axis activation. In a malignant context, SMAD2 and SMAD3 stimulate cell migration, and CRISPR-mediated SMAD2/3 knockout restricts pancreatic cancer invasiveness [127]. Hence, we summarize multiple studies demonstrating the significance of CRISPR systems in knocking out new targets in pancreatic cancer cells to identify their biological roles and examine their suppression effects in oncogenesis.

Clinical dataset analysis indicates that ankyrin repeat and PH domain 2 (ASAP2) exhibits elevated DNA copy numbers in 72.3% of PDAC patients, correlating with significantly worse prognoses. Fujii et al. utilized the CRISPR/Cas9 knockout method to elucidate the role of ASAP2 in tumor growth, aiming to identify further potential clinical targets. These findings suggest that ASAP2 may serve as a promising therapeutic target in PDAC [128]. Similarly, He et al. contributed to the identification of pancreatic

tumor drivers for future targeting research by demonstrating that the knockout of Rab11-family interacting protein 4 (Rab11-FIP4) via the CRISPR/Cas9 system resulted in the inhibition of cell cycle progression and tumorigenesis in PANC-1 cells. Rab11-family interacting proteins (Rab11-FIPs) could function as tumor promoters in certain cancers and exhibit elevated expression in 60 pancreatic cancer cases [129]. Moreover, immunohistochemical findings show elevated levels of Krüppel-like factor 5 (KLF5) in 96 PDAC samples and a murine model compared to controls. KLF5 might be essential for the formation of pancreatic intraepithelial neoplasia induced by oncogenic KRAS. It is noteworthy that acinar cells lose their acinar cell phenotype and adopt a duct cell phenotype during the reprogramming process towards PDAC. CRISPR/Cas9-mediated KLF5 knockout enhanced NDRG2 expression and diminished STAT3 activation, thereby decreasing acinar-to-ductal metaplasia and pancreatic intraepithelial neoplasia development in a murine model [130]. Amylase is also regarded as a marker for acinar cells, and its elimination leads to the progression of pancreatic cancer; CRISPR-mediated knockout of amylase results in diminished autophagy, thereby contributing to the etiology of pancreatic cancer [131].

CRISPR-based strategies could be designed to target multiple genes, including telomerase genes (e.g., TERT), alternative lengthening of telomeres (ALT) axis genes, and other critical genes involved in cancer cell survival [132]. In order to achieve immortalization, the majority of tumor cells use TERT as a mechanism to halt progressive telomere attrition [133]. Numerous studies employed Cas9 to edit promoter sequences or knock out the TERT gene [134–138]. A study employs a CRISPR/Cas9-mediated gene disruption strategy that utilizes three gRNAs to target three exons of the TERT gene in pancreatic, cervical, and breast cancer cells. This efficient editing strategy for TERT knockout reduces cancer cell survival both in vitro and in vivo [132]. Besides, the CRISPR/Cas9 genome editing tool reverses the epithelial to mesenchymal transition (EMT) through annexin A1 knockout, leading to a less aggressive phenotype. Moreover, this targeting reduces the metastasis rate in vivo, highlighting the significant oncogenic role of annexin A1 in the pancreatic cancer progression [139].

Recently, Zhang et al. utilize CRISPR/Cas9 to target CD73, resulting in the inhibition of cell growth and migration, as well as the induction of G1 cell cycle arrest in both human and murine cell lines. Furthermore, the ablation of CD73 resulted in the E-cadherin pathway activation and ERK/STAT3 signaling suppression [140]. To target genes linked to cancer development, Tsukumo et al. employed CRISPR systems to elucidate the effects of mammalian target of rapamycin (mTOR) inhibition on eIF4E-binding proteins (4E-BPs). In this study, the expression of 4E-BP3 was upregulated during extended mTORC1 inhibition at the transcriptional level, and CRISPR/Cas9-mediated knockout of the EIF4EBP3 gene promoter diminished the inhibitory effects of mTOR inhibitors, indicating that 4E-BP3 is a significant regulator of mTORC1 [141]. A study investigating the role of sphingosine kinases (SphK1 and SphK2) in pancreatic cancer revealed that CRISPR/Cas9-mediated knockout of SphK1 in PAN02 cells leads to enhanced proliferation and migration, reduced survival, and diminished lifespan in mice with experimental pancreatic cancer peritoneal carcinomatosis. Conversely, SphK2 knockout exhibits opposing effects that favor pancreatic cancer inhibition, positioning it as a potential target for PDAC treatment [142].

To target additional modulators with oncogenic roles in pancreatic cancer progression, which typically exhibit elevated levels in this context, several studies have focused on CRISPR/Cas9-mediated knockout of the RNA-binding protein HuR (ELAVL1), cadherin 17 (CDH17), leukemia Inhibitory Factor (LIF), and interleukin-2 receptor subunit gamma (IL2RG) to modify invasive characteristics [108, 143–146]. The detailed consequences of these knockouts are delineated in Table 2.

4.2 Correction and discovery of tumor suppressors

In the context of tumorigenesis, the inactivation of tumor suppressor genes is equally significant as the activation of oncogenes [171]. Loss-of-function mutations in the promoter regions of tumor suppressor genes, such as p16/CDKN2A, TP53, and SMAD4, are frequently observed in pancreatic carcinomas, with inactivation occurring in over 50% of all cases [147]. Repairing tumor suppressor genes aims to restore their normal functioning and activity, thereby inhibiting tumor occurrence and development, and CRISPR systems facilitate this objective [42]. For example, PTEN tumor suppressor expression is diminished or absent in a significant proportion of primary melanomas. The activation of PTEN expression via dCas9-VPR inhibited downstream oncogenic pathways, reduced cellular migration, and decreased colony formation in melanoma cells treated with various targeted therapy agents [172].

CRISPR systems have also opened new avenues in tumor suppressor discovery in relation to tumor treatment, and research has uncovered several favorable outcomes [42]. According to a study by Vorvis et al., pancreatic tumor xenografts with forkhead box protein A2 (FOXA2) deletion from the PANC-1 cell line had a noticeably higher weight and volume than the control tumors [147]. Similarly, ring finger protein 43 (Rnf43) is a tumor suppressor in the pancreatic cancer context, and its disruption as an E3 ubiquitin ligase by CRISPR/Cas9 accelerates $KRAS^{G12D}$-dependent tumor development [148]. Lastly, the inhibition of FAM110C and RAC1B through CRISPR/Cas9 knockout enhanced the PDAC cells invasiveness, suggesting its role as a potential tumor suppressor gene in PDAC [149, 150].

4.3 Overcoming drag resistance

Obviously, gradual emergence of drug resistance is a significant barrier in cancer therapy [173]. Since its discovery, CRISPR has been widely used to target drug resistance genes by directly editing genomic DNA, enabling phenotype modulation in cancer cells and identification of novel druggable targets [157, 174]. Although drug resistance is caused by a variety of mechanisms, the fundamental cause is typically an alteration in the genetic composition of tumor cells [175]. Numerous malignant cells exhibit elevated expression of mucin glycoproteins, including Mucin 4 (MUC4), and studies revealed that this overexpression is linked to drug resistance [176]. Skrypek et al. discovered that MUC4, expressed by pancreatic cancer cells, contributes to resistance against gemcitabine, an important chemotherapy agent utilized in the therapeutic regimes for pancreatic cancer patients [173, 177]. According to a recent study, CRISPR/Cas9-mediated knockout of MUC4 dramatically enhances pancreatic cancer cells' sensitivity to gemcitabine [151]. Additionally, germline BRCA mutations are identified in 4–7% of pancreatic cancer patients, and the presence of BRCA1 or BRCA2 mutations correlates with an elevated risk of developing various cancers, including pancreatic cancer [178]. Witz

Table 2 Recent studies on gene editing in pancreatic cancer employing CRISPR technology

Target	Gene function	Clinical relevance	Cell line/model	CRISPR type	CRISPR function	Effects	Ref.
KRASG12D	Oncogene	***	PANC-1, SUIT-2, the murine cell line TB32047	Cas9	Knockout	-Heterogeneously altered cell survival factors	[114]
KRASG12D	Oncogene	***	Cells isolated from KPC mouse model	Cas9	Knockout	-In vivo inhibition of tumor development	[115]
KRASG12D KRASG12C	Oncogene	***	AsPC-1, PANC-1 and HPAF-II	Cas13a	knockdown	-mRNA knockdown with 94% efficiency without affecting wild-type KRAS mRNA levels. -Increased apoptosis -Reduced tumor growth in vivo	[119]
KRASG12D	Oncogene	***	MIAPaCa-2, PANC-1	CasRx	knockdown	-Proliferation inhibition -Improvement in the Gemcitabine sensitivity -Inhibits the progression of patient-derived xenografts bearing mutant KRASG12D	[122]
SMAD2 SMAD3	Signaling mediator (oncogenic in PDAC)	***	BxPC-3	Cas9	Knockout	-Decreased migration ability -Invasiveness restriction	[127]
ASAP2	Oncogene	***	PANC-1, MIA PaCa-2	Cas9	Knockout	-Reduced cell proliferation -Decreased migration ability -Reduced colony formation in vivo and in vitro	[128]
Rab11-FIP4	Oncogene	**	PANC-1	Cas9	Knockout	-Inhibited cell growth, invasion and metastasis -Arrested cell cycle progression	[129]
KLF5	Oncogene	***	UN-KC-6141 (Mouse pancreatic cancer cell line derived from KC)	Cas9	Knockout	-Increased expression of NDRG2 -Reduced activation of STAT3 -Reduced acinar-to-ductal metaplasia and PanINs -Reduced cell proliferation	[130]
Amy2	Autophagy regulator	**	MIA PaCa-2, AR42J	Cas9	Knockout	-Decreased autophagy	[131]
TERT	Telomerase catalytic subunit / Immortalization	***	PANC-1	Cas9	Knockout	-Lowered telomerase activity -Decreased cell proliferation in vitro -Increased apoptosis in vitro -Reduced tumor growth in vivo	[132]

Table 2 (continued)

Target	Gene function	Clinical relevance	Cell line/model	CRISPR type	CRISPR function	Effects	Ref.
ANXA1 (Annexin A1)	Oncogene / EMT regulator	***	MIA PaCa-2	Cas9	Knockout	-Reduced migration and invasion ability -Increased apoptosis -Induced S/G2-phase cell cycle arrest -Decreased metastatic potential in vivo	[139]
NT5E (CD73)	Ectonucleotidase / Tumor growth and migration regulator	***	PANC-1, the murine cell line TB32047	Cas9	Knockout	-Inhibited cell growth and migration -Induced G1-phase cell cycle arrest -Inhibited cell migration In vitro -Reduced phosphorylation of ERK and STAT3 -Increased E-cadherin expression	[140]
EIF4EBP3	mTOR pathway regulator / Translational repressor	**	MIAPaCa-2, PANC-1	Cas9	Knockout	-Induced translation and proliferation during prolonged mTORC1 inhibition	[141]
SphK1	Lipid kinase / Oncogene	**	PAN02 murine pancreatic cancer cell line	Cas9	Knockout	-Increased proliferation and migration -Reduced survival in mouse model	[142]
SphK2	Lipid kinase / Tumor suppressor-like role	***	PAN02 murine pancreatic cancer cell line	Cas9	Knockout	-Decreased proliferation and migration -Increased survival in mouse model	[142]
ELAVL1 (HuR)	RNA-binding protein / Post-transcriptional regulator	***	MIAPaCa-2	Cas9	Knockout	-Increased apoptosis -Inhibition of the PDX xenograft growth	[144]
CDH17	Cell adhesion molecule / Oncogene	**	Panc02-H7	Cas9	Knockout	-Reduced tumor growth in vitro and in vivo -Reduced clonogenic survival -Suppressed motility -Increased lifespan in mouse model	[143]
LIF	Cytokine / Tumor progression factor	**	Panc1.0	Cas9	Knockout	-Repressed sphere-forming ability -Enhanced overall survival rate in FVB/n mice	[108]
IL2RG	Cytokine receptor subunit / Immune modulator	**	bkpc58 and TB32043 (Mouse pancreatic cancer cell line derived from KPC)	Cas9	Knockout	-Reduced tumor growth in vivo -Reduced JAK3 expression	[145]

Table 2 (continued)

Target	Gene function	Clinical relevance	Cell line/model	CRISPR type	CRISPR function	Effects	Ref.
FOXA2	Tumor suppressor	**	PANC-1	Cas9	Knockout	-Increased tumor growth in vivo	[147]
Rnf43	Tumor suppressor	***	ESCs	Cas9	Knockout	-Increased tumorigenesis in vivo -Reduced survival in mouse model	[148]
RAC1B	Tumor suppressor	**	PANC-1	Cas9	Knockout	-Enhanced migration -Upregulation of the expression of ALK2 -Elevated the phosphorylation of SMAD1/5	[149]
FAM110C	Tumor suppressor	**	Panc10.05	Cas9	Knockout	-Increased proliferation in vitro -Increased invasion in vivo -Enhanced VE-822 and MK-8776 sensitivity	[150]
MUC4	Mucin glycoprotein / Promotes drug resistance	***	T3M4	Cas9	Knockout	-Reduced migration and invasion -Reduced tumor growth, proliferation, and angiogenesis -Increased apoptosis -Enhanced gemcitabine sensitivity -Reduced PDAC cell tumorigenicity in vitro and in vivo	[151]
BRCA1 BRCA2	DNA repair genes / Tumor suppressors	***	Capan-2, T3M4	Cas9	Knock in	-Decreased BRCA protein levels -Enhanced Olaparib sensitivity -Increased apoptosis after treatment by Olaparib	[152]
GPRC5a	Oncogenic signaling	**	MIA PaCa-2 and Mouse pancreatic cancer cell line TB32047	Cas9	Knockout	-Reduced the proliferation and migration -Enhanced gemcitabine, oxaliplatin, and fluorouracil sensitivity -Upregulated GSK-3β phosphorylation	[153]
GALNT3	Glycosylation enzyme	**	Capan-1	Cas9	Knockout	-Reduced tumorigenesis in vitro -Reduced clonogenicity -Reduced migration -Reduced expression of self-renewal markers (SOX2 and OCT3/4)	[154]

Table 2 (continued)

Target	Gene function	Clinical relevance	Cell line/model	CRISPR type	CRISPR function	Effects	Ref.
ISG15	Interferon-stimulated gene; ISGylation factor	***	PDAC PDX-derived cultures Panc185 and Panc354	Cas9	Knockout	-Reduced overall ISGylation -Altered mitochondrial state and metabolism -Altered cancer stem cell markers -Reduced self-renewal -Reduced mitophagy -Increased autophagy -Enhanced autophagy and mitochondrial respiration inhibitors sensitivity -Reduced metabolic plasticity -Reduced tumorigenesis in vivo	[155]
HIF-1α	Hypoxia response regulator / transcription factor	***	BxPC-3	Cas9	Knockout	-Reduced metastasis in vitro and in vivo -Enhanced the antiproliferation effect of paclitaxel	[156]
HO-1	Antioxidant enzyme induced by hypoxia	**	Capan-1	Cas9	Knockout	-Increased apoptosis under hypoxia -Reduced proliferation -Enhanced gemcitabine sensitivity	[157]
TP53	Tumor suppressor / cell cycle regulator	***	Mouse pancreatic cancer cell Panc02	Cas9	Knockout	-Increased aggressive traits in acidic TME	[158]
TP53	Tumor suppressor / cell cycle regulator	***	primary murine pancreatic ductal epithelial cells	Cas9	Knock in	-Increased neutrophil infiltration -Reduced the presence of CD3 + T cells, CD8 + T cells, and CD4 + T helper 1 cells	[159]
ISG15	Ubiquitin-like modifier involved in immune modulation	***	Mouse pancreatic cancer cell Panc02	Cas9	Knockout	-Reduced PDL-1 expression -Increased PD-1 treatment efficacy in vivo -Increased number of CD8 + tumor-infiltrating lymphocytes in vivo -Reduced Tregs number in vivo -Reduced tumor cell growth in vivo	[160]
PCK1	Key gluconeogenic enzyme (PEP production)	***	Primary pancreatic cancer cells	Cas9	Knockout	-Reduced cell growth, proliferation, migration and invasion Increased apoptosis	[161]
C1GALT1	Core 1 β1,3-galactosyltransferase (glycosylation)	**	T3M4, Colo-357 and BxPC-3	Cas9	Knockout	-Increased truncation of O-glycosylation on MUC16 -Increased proliferation -Increased tumor cell growth and migration -Increased metastasis and tumorigenicity	[162]

Table 2 (continued)

Target	Gene function	Clinical relevance	Cell line/model	CRISPR type	CRISPR function	Effects	Ref.
GCNT3	Glycosyltransferase regulating β-catenin/MUC4 signaling	***	HPAF/CD18	Cas9	Knockout	-Reduced progression and metastasis -Downregulated cell cycle genes and β-catenin/MUC4 axis	[163]
ISL2	Tumor suppressor	***	PDX366, AsPC1	Cas9	Knockout	-Increased tumor development in vivo	[164]
ISL2	Tumor suppressor	***	KP4	dCas9	Activation	-Reduced proliferation	[164]
miR-3064	Aggressiveness regulator	***	PaCa-2	Cas9	Knockout	-Reduced the aggressive phenotypes of PC cells -Reduced tumor cell growth in vivo	[165]
miR-21	Tumor promoter	***	PANC-1, MiaPaCa-2	Cas9	Knockout	-Reduced proliferation -Enhanced PD-0332991 sensitivity -Induced cell cycle arrest in combination with PD-0332991 -Reduced migration in combination with PD-0332991	[166]
HOXA-AS3	miRNA sponge/cell cycle regulator	***	PANC-1, Bxpc-3	dCas9	Repression	-Reduced proliferation -Reduced tumor cell growth in vivo	[167]
Ring1b	Epigenetic repressor (H2AK119ub1 writer)	**	Cells isolated from KPC mouse model	Cas9	Knockout	-Impaired acinar cell dedifferentiation -Reduced tumor formation	[168]
TP53	Tumor promoter	***	MRC5 cells	dCas9	Repression	-Reduce BHLHA15 differentiation marker	[169]
MIR100HG	Enhancer of lncRNA gene MIR100HG	**	PANC-1	dCas9	Repression	-Downregulation of MIR100HG -Reduced TGF-β pathway -Reduced KRAS, p53, MTOR and TNFα signaling	[170]

The level of clinical significance is indicated by stars based on the strength and translational potential of the reported phenotypes

CSC cancer stem cell, *PC* pancreatic cancer

**Moderate clinical relevance; ***high clinical relevance

et al. demonstrated that the insertion of Glu255 and Cys711 mutations via CRISPR/Cas9 results in the generation of truncated BRCA1 and BRCA2 proteins, respectively, and enhanced olaparib sensitivity [152]. Similarly, the ablation of GPRC5a (G-protein-coupled receptor family C, member 5, group A) as an overexpressed gene in PDAC by CRISPR/Cas9 mitigated drug resistance to gemcitabine, oxaliplatin, and fluorouracil in both human and murine pancreatic cancer cell lines [153].

4.4 Targeting genes associated with cancer stem cells

The CRISPR system is also applicable for the elimination of cancer stem cells through its gene-editing potential [155]. Using the CRISPR system, Barkeer et al. assess the significance of glycosylation in pancreatic cancer stem cells, given its crucial role in the aggressiveness of pancreatic cancer. The CRISPR/Cas9-mediated silencing of Polypeptide N-Acetylgalactosaminyltransferase 3 (GALNT3) in Capan1 cells led to a diminished expression of self-renewal markers [154]. Moreover, Alcalá et al. reported that PDAC stem cells have elevated levels of interferon-stimulated gene 15 (ISG15) expression and protein ISGylation, which are crucial for sustaining their metabolic plasticity. According to this study, CRISPR-mediated ISG15 genomic editing downregulates total ISGylation, which hinders pancreatic cancer cells' ability for self-renewal and tumorigenic capacity [155].

Cancer stem cells drive tumor heterogeneity and the eradication of these stem-like tumor cell subpopulations could be the most effective approach in cancer therapy. However, the emerging notion that non-cancer stem cells can transmute into cancer stem cells suggests that this strategy is probably going to lose its potency [179, 180], and cancer stem cell plasticity facilitates adaptation to intrinsic and extrinsic damages or stimuli [181].

4.5 Modulation of TME and cancer immunity

The hallmark traits of cancer include resistance to apoptosis, induction of angiogenesis, facilitation of invasion and metastasis, tumor-promoting inflammation, and evasion of immune surveillance. All of these traits are shaped to varying degrees by elements within the tumor microenvironment (TME). This complex and heterogeneous environment poses significant challenges to therapeutic efficacy. In PDACs, tumors are often poorly vascularized. This is largely due to the presence of dense desmoplastic stroma, which sequesters and inactivates pro-angiogenic signals and thus hinders the formation of new blood vessels [182].

In addition, accelerated cellular proliferation, a characteristic hallmark of oncogenesis, necessitates elevated oxygen utilization, resulting in a hypoxic microenvironment within the tumor that displays certain aberrant alterations [183, 184]. In an oxygen-deficient tumor microenvironment, hypoxia-inducible factor-1 alpha (HIF-1α) supports tumor cells' adaptation to hypoxia by acting as a key regulator of the cellular response to variations in oxygen levels [185]. In fact, the regulatory effects of HIFs in response to hypoxia promote malignant PDAC phenotypes; thus, targeting HIFs and associated signaling pathways could enhance pancreatic cancer treatment [186]. An in vivo study conducted by Li et al. showed that the CRISPR/Cas9 delivery system made of R8-dGR-modified cationic liposomes makes the BxPC-3 pancreatic cancer model susceptible to the paclitaxel effect by downregulating HIF-1α and its downstream molecules VEGF and

MMP9. Additionally, given that CRISPR/Cas9 minimally influenced HIF-1α expression in normal hepatic cells, this engineered liposomal delivery did not elicit significant toxicity in vivo [156]. Furthermore, hypoxia mediates numerous effects by upregulating various mediators such as heme oxygenase-1 (HO-1), which may support pancreatic cancer cells under oxidative stress. Abdalla et al. demonstrated that targeting HO-1 in PDAC cells, through various methods including the CRISPR/Cas9 knockout system, results in a greater gemcitabine sensitization in pancreatic cancer cell lines [157].

As implied previously, the inactivation of the tumor suppressor p53 is correlated with higher invasiveness and a poorer prognosis. In the recent study conducted by Czaplinska et al., the gain of cancerous features caused by acid adaptation was further augmented by the TP53 CRISPR knockout, intensifying the aggressive characteristics of PDAC cells in acidic tumor microenvironments [158]. Moreover, a correlation exists between TP53 mutations and neutrophils, due to the fact that the human PDAC squamous subtype demonstrated a notable enrichment of tumor-associated neutrophil infiltration [187]. To elucidate this effect on PDAC, Siolas et al. employed the CRISPR/Cas9 system alongside a single-strand DNA HDR template to introduce the $TP53^{R172H}$ mutation into murine pancreatic ductal epithelial cells originating from a $KRAS^{G12D}$ mutant GEMM. The introduction of the $TP53^{R172H}$ mutation resulted in heightened neutrophil infiltration and decreased T-cell presence [159].

A hallmark of PDAC is the fibrotic stroma, which contributes to an immune-privileged microenvironment, mainly due to limited immune cell infiltration [188]. This condition arises from the dense desmoplastic stroma, which is composed of activated pancreatic stellate cells and extracellular matrix. These components create a physical barrier that prevents effector T cells from accessing the tumor core [189]. As PDAC progresses, the tumor-infiltrating T cell profile shifts toward reduced CD8 + cytotoxic T cells and an increased proportion of CD4 + regulatory T cells (Tregs). This imbalance, along with the scarcity of activated or functional CD8 + T cells in the tumor microenvironment, reflects a state of immune exhaustion and impaired antitumor response. Moreover, the PDAC microenvironment is populated by immunosuppressive myeloid cells—including tumor-associated macrophages (TAMs), neutrophils, and myeloid-derived suppressor cells (MDSCs)—which secrete cytokines such as IL-10 and TGF-β that further inhibit T cell cytotoxicity. Collectively, these factors establish an immune-privileged niche that enables tumor cells to evade immune surveillance and supports their continued growth [190]. As such, without strategies specifically addressing this privileged status of PDAC, CRISPR-based immunomodulatory interventions may fail to achieve durable antitumor responses in these tumors. In PDAC, CRISPR-mediated attenuation of oncogenic KRAS does not simply reduce intrinsic tumorigenicity. Instead, it disrupts KRAS-driven mechanisms of immune suppression and reveals a tumor cell reliance on KRAS signaling to evade immune surveillance. KRAS-deficient cells are more effectively cleared in immunocompetent hosts, highlighting that CRISPR editing may unmask intrinsic immune vulnerabilities in PDAC rather than directly increasing therapeutic efficacy [191].

A logical approach in PDAC treatment is ameliorating the immunosuppressive TME. The CD40-CD40L signaling pathway is identified as a promising target in the PDAC immunotherapy, as it activates antigen-presenting cells (APC) through the NF-κB pathway, improves the recruitment of immune effectors, and mobilizes endogenous tumor-recognizing T cells. In 2022, Wang et al. were the first to utilize the intratumoral

administration of CRISPR/Cas9-mediated CD40L-armed oncolytic therapy. They used CRISPR/Cas9-based gene editing to incorporate the membrane-bound form of murine CD40L into oncolytic herpes simplex virus-1. By this approach, the mice model survival is considerably extended due to altered TME with higher DC maturation and DC-dependent activation of cytotoxic T cells. Additionally, increased TME's pro-inflammatory macrophages and lymphocytes improve sensitivity to immunotherapy and produce a long-lasting endogenous immune response [192, 193]. Furthermore, CRISPR-mediated ISG15 knockdown led to an increased population of CD8 + tumor-infiltrating lymphocytes, reduced tumor programmed death ligand-1 (PD-L1) expression, resulting in an enhanced efficacy of anti-programmed cell death protein-1 (PD-1) therapy [160].

4.6 Targeting cancer metabolism

CRISPR tools have extensive applications in regulating cancer metabolism and targeting enzymes within tumor metabolic pathways. Phosphoenolpyruvate carboxykinase (PCK) is the essential and rate-limiting enzyme in gluconeogenesis that catalyzes the conversion of oxaloacetate and GTP into phosphoenolpyruvate (PEP), GDP, and CO_2 [194]. PCK1 exhibited a significant increase in human pancreatic cancer tissues and cells, and Zhu et al. indicate that its ablation using CRISPR or shRNA techniques inhibited characteristics that promoted tumor invasion and triggered substantial apoptosis [161]. Moreover, CRISPR/Cas9-based knockout systems have demonstrated the significance of glycosylation in pancreatic cancer. In human PDAC cells, the CRISPR-mediated deletion of C1GALT1 enhances the aberrant glycosylation of MUC16, thereby increasing tumorigenicity and potentially contributing to tumor aggressiveness in a mouse model [162]. Gupta et al. also discovered that the knockdown of GCNT3 as a specific glycosyltransferase inhibits the growth and metastasis of pancreatic cancer by inhibition of the β-catenin/MUC4 pathway [163].

4.7 Modulation of non-coding regions

Noncoding regions constitute 98% of the human genome and encompass the majority of cancer-related somatic mutations [195]. Targeting these mutations, which genetically distinguish malignant cells from normal cells, could serve as a cancer therapeutic approach. Unfortunately, most targeted therapies, as well as engineered vaccines and synthesized agents, concentrate on mutations in coding regions, which obviously have a limited number [196]. In 2024, Teh et al. use CRISPR/Cas9 knockout to create DSB in three pancreatic cancer cell lines at the sites of somatic mutations within noncoding regions that create PAMs. This CRISPR system selectively induces 69–99% cell death in targeted cells utilizing 4–9 sgRNAs customized based on the somatic PAM discovery findings [197].

Additionally, a class of RNA molecules found in the transcriptome known as noncoding RNAs is crucial for influencing the biological characteristics of different types of malignancy and controlling gene expression [198]. Hence, the knockdown of miRNAs may enhance chemotherapy resistance and inhibit tumor growth, leading to improved cancer therapy [42]. The CRISPR/Cas9 system utilizes a specifically designed gRNA to direct Cas9 in generating DSB at the target sites within miRNA genes. According to Zhao et al.'s research, the carcinogenic miRNAs miR-21 and miR-30a can be silenced through this methodology [199]. CRISPR/Cas9-mediated miRNA targeting presents

significant therapeutic potential for pancreatic cancer. Yan et al. demonstrated that CRISPR/Cas9-mediated genome editing of miR-3064 in PaCa-2 cells attenuated aggressive phenotypes in vitro and inhibited tumor growth in xenograft mouse models, suggesting that miR-3064 may serve as a viable target for pancreatic cancer therapy [165]. The recent study by Mortoglou et al. demonstrated that miR-21 knockout via CRISPR/Cas9 synergically improved PD-0332991 treatment as a CDK4/6 inhibitors, and indicated a novel approach for PDAC treatment [166]. Long non-coding RNAs (lncRNAs), another category of noncoding RNAs, have been demonstrated to influence cancer development. To prevent mRNA degradation, lncRNA HOXA-AS3 functions as a miRNA sponge [200], and its upregulation results in the proliferation and progression of several cancer types [201]. In the context of PDAC, HOXA-AS3, by inhibiting the miR-29c/CDK6 axis, promotes the proliferation of malignant cells. Zhang et al. suppressed HOXA-AS3 using CRISPR/dCas9. In this setting, CDK6 mitigates the effects of HOXA-AS3 ablation, thereby inhibiting the progression of pancreatic cancer [167]. However, silencing of essential lncRNAs in pancreatic cancer cells does not always produce a significant phenotypic effect, likely due to functional redundancy within the non-coding transcriptome [202].

Additionally, the primary challenge in miRNA targeting via CRISPR/Cas is the short sequence of miRNA, which complicates the detection of sgRNAs and PAM sequences [203]. Furthermore, indels generated by the CRISPR/Cas system in non-coding regions do not always result in gene loss of function due to the absence of an open reading frame. So, it makes the ablation of non-coding genes more challenging. To address this challenge, Ho et al. inhibit the NHEJ pathway and effectively knock out miR-21, miR-29a, lncRNA-21 A, AK023948, and UCA1 in cancer cells via homologous recombination (HR). In this study, two sgRNAs specifically induced DSBs at the desired sites and cleaved the large fragment [204].

4.8 Epigenetic regulation

In addition to other factors, multiple epigenetic alterations that can promote carcinogenesis and drug resistance are critical characteristics of cancer. Nowadays, epigenome editing—particularly through the CRISPR system—makes it possible for the analysis of precise epigenetic changes and their impact on the development and treatment of cancer [48]. Basically, certain lysine residues on histones can be methylated, acetylated, or ubiquitinated by epigenetic mechanisms to control DNA accessibility, chromatin compaction, and gene expression patterns that determine cell identity and differentiation [205]. Epigenetic mechanisms are a crucial factor in the metastasis of cancer cells in pancreatic cancer. In pancreatic cancer cells and acinar-to-ductal metaplasia, Ring1b catalyzes the monoubiquitylation of lysine 119 on histone H2A, a crucial epigenetic alteration that epigenetically silences acinar cell transcription factors. CRISPR-mediated knockout of ring1b in a mouse model significantly hindered acinar cell dedifferentiation, thereby promoting tumor cell reprogramming in favor of an epigenetically modified, less aggressive phenotype [168]. Meanwhile, in a $KRAS^{G12D}$-driven murine model of PDAC, CRISPRi-mediated downregulation of p53 decreased differentiation markers [169].

In addition, as a result of the capability of the dCas9-effector system to either activate or repress endogenous gene expression, a new opportunity has been presented for the further investigation of cancer-associated non-coding mutations [48]. Non-coding

mutations that affect enhancer cluster function are a contributing factor to cancer. In the study conducted by Patel et al., the downregulation of MIR100HG, which is accompanied by a substantial reduction in the TGF-β pathway and other PDAC critical pathways, is the result of CRISPRi disruption of an enhancer cluster that harbors non-coding mutations over the long non-coding RNA gene MIR100HG [170].

Finally, it is noteworthy to imply that epigenetic altering approaches are reversible, which eliminates the risk of inducing sequence modifications to the target DNA, a critical factor in the targeting of tumors with high levels of genetic instability, in contrast to Cas9 genome engineering, which invariably leads to permanent changes [206]. Additionally, the longevity of the epigenetic and transcriptional modifications that dCas9 editing induces may be contingent upon the targeted loci and the specific combination of effectors. Therefore, adapting the technology for the manipulation of various loci in various cell types with varying chromatin microenvironments is a challenge facing current epigenome engineering research [207].

4.9 Gene modification through base and prime editing

Cas9-induced DSBs are repaired via NHEJ or HDR, but HDR's low efficiency and restriction to dividing cells have shifted focus toward base and prime editors enabling precise, donor-free edits. DNA base-editing tools comprise cytosine base editors (CBEs), which possess cytosine deamination capability that converts C: G to T: A base pairs, and adenine base editors (ABEs), which exhibit adenosine deamination effects that alter A: T to G: C base pairs [208]. To evaluate the efficiency and specificity of CRISPR/Cas9 base editing in targeting mutations, Sayed et al. utilized ABEs to cleave or correct different oncogenic KRAS variants and TP53 mutations in several cancer cells such as PANC-1 and pancreatic cancer organoids [209].

Although base editors, such as ABE and CBE, were designed to introduce precise nucleotide substitutions at the DNA level, increasing evidence indicates that they can also cause unintended edits in RNA molecules. These off-target effects, especially in the context of solid tumors, may lead to unpredictable alterations in both coding and non-coding transcripts. Such alterations can include missense and nonsense mutations, disruptions at splice junctions, and modifications in regulatory untranslated regions (5′ and 3′ UTRs). Collectively, these findings suggest that base editor–induced off-target events are often more complex and widespread compared to those observed with nuclease-based genome editing, posing additional challenges for their safe and effective use in therapeutic settings [210].

In addition, the primary drawback of the existing base-editing technologies is their incapacity to execute insertions, deletions, and the majority of transversions, as well as their inability to produce precise base-edits beyond the four transition mutations (C to T and A to G) [211]. To address these deficiencies, prime editors (PEs) as an innovative method target small insertion and deletion as well as both transition and transversion mutations. This system does not depend on DSBs or donor DNA; instead, it utilizes an engineered reverse transcriptase (RT) fused to Cas9 nickase and a pegRNA as its primary components [208]. This pegRNA markedly differs from conventional sgRNAs by incorporating an additional sequence that determines the desired sequence alterations, alongside a complementary sequence in the 5′ end that directs nCas9 to its target sites [123]. In this strategy for altering the target region, Cas9 creates a primer for the RT by

nicking the unbound PAM-containing strand. Then, the cleaved strand is subsequently elongated by the RT utilizing the interior sequence of the pegRNA as a template [208]. The PE system created by Jang et al. as a "one-to-many" therapeutic strategy for correcting KRAS oncogene variants consists of universal pegRNAs with a high correction efficiency, capable of correcting 12 distinct KRAS mutations, encompassing all G12 and G13 variants in pancreatic cancer cells [212].

5 Clinical application of CRISPR-based therapies for pancreatic cancer

CRISPR gene-editing technology has made significant strides in recent years, advancing into clinical applications for a range of cancer treatments, with several trials currently investigating its potential. CRISPR-based therapies for pancreatic cancer are still in early preclinical stages, with no direct clinical trials yet; however, ongoing studies in solid tumors may provide valuable insights into their safety, efficacy, and future integration into pancreatic cancer treatment. For example, a Phase I/II trial (NCT04426669) by Intima Bioscience uses CRISPR/Cas9 to knock out the Cytokine-Induced SH2 Protein (CISH) gene in tumor-infiltrating lymphocytes (TILs) in metastatic gastrointestinal cancers. CISH acts as an intracellular immune checkpoint, and its disruption is expected to enhance T cell antitumor activity [213]. The aim of this study is the knockout of CISH to enhance the ability of TILs and T cells to target and destroy tumors in patients with metastatic pancreatic and other gastrointestinal cancers as well as assess the safety and efficacy of this strategy in human patients. In 2023, CRISPR Therapeutics initiated a Phase I/II clinical trial (NCT05795595) to evaluate the safety and efficacy of anti-CD70 allogeneic CRISPR/Cas9-engineered T cells (CTX131) in patients with relapsed or refractory solid tumors, including pancreatic adenocarcinoma [214]. Preliminary results have demonstrated positive safety profiles and a dose-dependent effect on disease activity.

Moreover, multiple solid tumors frequently express mesothelin on their surface, including pancreatic cancer, and a Phase I clinical trial (NCT03545815) employed CRISPR/Cas9 to knock out PD-1 and the T cell receptor (TCR) in mesothelin-specific CAR T cells (MPTK-CAR-T) for patients with mesothelin-positive solid tumors. Similarly, another Phase I study (NCT03747965) investigated the impact of PD-1 knockout in CAR T cells, combined with paclitaxel and cyclophosphamide pretreatment. Both studies demonstrated preliminary safety and feasibility; however, further investigations are required to evaluate their broader clinical potential [215]. These clinical trials may also hold therapeutic relevance for pancreatic cancer.

While such advancements offer significant promise, it is important to recognize that many CRISPR-based cancer therapies remain in early-phase clinical trials. Continued research is essential to establish their safety, efficacy, and potential role within standard cancer treatment regimens. Moreover, ongoing efforts to develop novel CRISPR-based strategies specifically targeting pancreatic cancer are crucial to overcoming current therapeutic challenges.

6 Limitation and challenges in crispr/cas systems application

One of the major issues regarding CRISPR/Cas technology is off-target effects, whereby the system may edit genes other than the actual target. These unwanted changes could destroy normal gene function and cause cancerous transformations to occur [216]. For example, off-target changes in tumor silence genes or activation of oncogenes can drive

the expansion of cancer [217]. Additionally, studies have suggested that CRISPR-induced DSBs can lead to chromosomal rearrangements and genomic instability, further increasing the risk of cancer [218]. Such unintended genetic modifications may have severe consequences, particularly in therapeutic applications. To mitigate and reduce off-target effects, a variety of strategies have been established, including the application of high-fidelity Cas9 variants, which are specially engineered forms of the Cas9 enzyme. that have been modified to reduce off-target activity while maintaining on-target efficiency [31].

On the other hand, CRISPR/Cas9-mediated gene disruption can unintentionally trigger a DNA damage response via activation of the p53 signaling pathway. This often results in growth arrest or apoptosis of edited cells, particularly in tumors with functional p53, thereby reducing the overall therapeutic efficacy before the desired genetic modification can exert its intended effect [49].

The delivery of CRISPR/Cas systems is critical in their success rate in gene editing tasks. There are three major forms of delivery: sgRNA and mRNA of Cas protein, plasmid DNA encoding Cas proteins together with sgRNA, and the Cas/sgRNA RNP complex. All of them come with their respective challenges and limitations. For example, mRNA facilitates the translation of sgRNA in the cytoplasm. While this is feasible, its practical application is constrained by the relatively short stability of the RNA and degradation mechanisms. Plasmid DNA will be more stable once transfected, but the requirement for nuclear entry will reduce the efficiency of Cas9 generation. The Cas/sgRNA RNP complex does allow faster genome editing and less off-target damage, but delivering the RNPs is often difficult due to the large size of the Cas enzyme [219, 220].

Physical delivery methods encompass microinjection, electroporation, and hydrodynamic injection, which facilitate the direct introduction of CRISPR/Cas components into cells through various techniques. Microinjection is a very precise method but is impractical for large-scale applications because it is very labor-intensive. Electroporation uses electrical pulses to create temporary pores in cell membranes and can achieve high delivery efficiency but may cause cell death and loss of cell stemness. Hydrodynamic injection, though effective in some animal models, is associated with organ trauma and physiological complications, which make it less suitable for clinical applications [221].

The viral and non-viral delivery methods also have their own unique challenges. Viral vectors, including adeno-associated viruses (AAVs), adenoviruses (AdVs), and lentiviruses, are indeed efficient while capable of provoking immune responses and have size limitations for the genetic material they could carry. Non-viral methods include lipid nanoparticles and polymer nanoparticles, which have lower immunogenicity and potential for targeted delivery but are facing issues with stability, efficiency, and potential toxicity. Improvements in these delivery technologies are key to enhancing the safety and efficacy of CRISPR/Cas-based therapies [222]. Furthermore, exosomes have emerged as an efficient non-viral delivery system. This method offers a promising and reliable approach to delivering CRISPR/Cas systems, especially in areas where other delivery methods have faced limitations [115].

Prolonged expression of bacterial Cas9 protein in mammalian cells poses significant challenges for therapeutic applications, primarily due to increased risks of off-target genome editing, cytotoxicity, and activation of host immune responses. Delivery of CRISPR/Cas9 via AAVs, although promising for gene therapy, is limited by its

immunogenicity and payload capacity. Recent studies demonstrate that AAV-CRISPR/Cas9 induces distinct cellular and molecular host immune responses without causing extensive cellular damage in vivo [223]. Importantly, investigations into human serum revealed preexisting adaptive immune responses against commonly used Cas9 orthologs derived from Staphylococcus aureus (SaCas9) and Streptococcus pyogenes (SpCas9), indicating that a significant proportion of individuals possess antibodies and antigen-specific T cells targeting these bacterial proteins [224]. These findings highlight a prevalent humoral and cell-mediated immunity to Cas9 that could potentially reduce editing efficacy and trigger inflammation or clearance of edited cells in clinical settings. Therefore, immunogenicity remains a critical barrier that must be addressed to enable safe and effective clinical translation of CRISPR/Cas9-based therapies.

Biocompatibility is a prime concern for clinical applications of the CRISPR/Cas systems. The components that make up these systems—the Cas proteins and delivery vectors—have to be biocompatible with the host organism to minimize the adverse resistant reaction and toxicity [225]. Key contemplations involve the advancement of biocompatible conveyance strategies, including lipid nanoparticles and polymer-based systems, capable of delivering CRISPR/Cas components with high efficiency and low immunogenicity/toxicity [226].

The ethical and moral implications of CRISPR/Cas technology are major and complex. Key issues to be taken into consideration are the possible off-target effects, which would lead to unwanted genetic modification, and concerns regarding safety and long-term effects of gene editing [227]. Full regulatory frameworks and clear ethical guidelines are, therefore, necessary to regulate responsible use of CRISPR/Cas technology, oversight of research and clinical use to prevent misuse, and protection of human rights [228].

Taken together, the successful translation of CRISPR from experimental studies to routine clinical use will require overcoming substantial challenges, including optimizing delivery mechanisms, minimizing off-target effects, and addressing ethical concerns surrounding genetic modifications in human patients.

7 Future direction

The rapid evolution of CRISPR/Cas technology holds transformative potential for pancreatic cancer research and therapy. One critical avenue is the refinement of delivery systems to enhance precision and biocompatibility. Innovations in non-viral vectors, such as lipid nanoparticles and exosomes, may overcome current limitations in stability and immunogenicity, enabling safer in vivo applications [115, 219–222]. Additionally, the development of high-fidelity Cas variants and novel editing tools, including base and prime editors, could minimize off-target effects while enabling precise correction of oncogenic mutations like KRAS [35–37, 208, 212].

Combining CRISPR-mediated therapies with conventional treatments, such as chemotherapy or immunotherapy, may synergistically enhance efficacy. For instance, CRISPR-edited CAR T-cells or oncolytic viruses armed with immune modulators could remodel the immunosuppressive tumor microenvironment [93, 94]. However, there is a significant lack of research in the application of CRISPR for immunotherapy specifically for pancreatic cancer. This gap is particularly notable in the development of CRISPR-based CAR-T cell therapies, overcoming the immune suppression mechanisms in pancreatic

tumors. These areas need more focused investigation for better treatment strategies for pancreatic cancer.

Furthermore, patient-derived organoids and GEMMs will remain indispensable for validating therapeutic targets and tailoring personalized regimens, particularly for rare genetic subtypes [60, 61].

Expanding CRISPR screens to non-coding genomic regions and epigenetic regulators could uncover novel vulnerabilities, such as enhancer clusters or lncRNAs, which drive tumor plasticity and resistance [195, 203].

8 Conclusion

In pancreatic cancer research, advanced model systems such as GEMMs and immunocompetent organoids have provided essential platforms for studying tumor biology and therapeutic responses. These models replicate the complex microenvironment and genetic landscape of pancreatic tumors. Building on these models, CRISPR-based functional screening has enabled high-throughput identification of critical vulnerabilities. Through diverse CRISPR platforms—from Cas9 to base and prime editing—researchers have elucidated the roles of genomic elements or directly edited them, expanding our understanding of pancreatic tumor biology and opening new avenues for targeted therapies.

Despite progress, significant challenges must be overcome before clinical application. Identifying the most critical gene targets is essential, alongside improving the accuracy, efficiency, and safety of CRISPR techniques to meet clinical standards. Notably, unintended activation of the p53 pathway by Cas9 may promote the selection of p53-inactivating mutations, raising further safety concerns. Overcoming these obstacles requires rigorous standardization of protocols, development of more effective delivery systems, and thorough validation. Future research should focus on refining delivery methods, enhancing editing specificity, and integrating CRISPR with complementary technologies such as single-cell genomics and artificial intelligence to develop personalized therapies. Robust trials and ethical oversight are crucial to ensuring safe, effective, and accessible treatments.

Ultimately, through multidisciplinary collaboration and continued innovation, CRISPR-based approaches hold great promise to transform pancreatic cancer from a lethal malignancy into a manageable disease, offering new hope to patients worldwide.

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