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REVIEW

Improving the prognosis of pancreatic cancer: insights from epidemiology, genomic alterations, and therapeutic challenges

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Abstract **Pancreatic cancer, notorious for its late diagnosis and aggressive progression, poses a substantial challenge owing to scarce treatment alternatives. This review endeavors to furnish a holistic insight into pancreatic cancer, encompassing its epidemiology, genomic characterization, risk factors, diagnosis, therapeutic strategies, and treatment resistance mechanisms. We delve into identifying risk factors, including genetic predisposition and environmental exposures, and explore recent research advancements in precursor lesions and molecular subtypes of pancreatic cancer. Additionally, we highlight the development and application of multi-omics approaches in pancreatic cancer research and discuss the latest combinations of pancreatic cancer biomarkers and their efficacy. We also dissect the primary mechanisms underlying treatment resistance in this malignancy, illustrating the latest therapeutic options and advancements in the field. Conclusively, we accentuate the urgent demand for more extensive research to enhance the prognosis for pancreatic cancer patients.**

Keywords pancreatic cancer; cancer screening; single cell; molecular alterations; precancerous lesion; therapy resistance

Introduction

Pancreatic cancer is a malignancy with a particularly poor prognosis with a 5-year survival rate of approximately 12%. It is projected to ascend as the second leading cause of cancer-related mortality in the United States by 2040 [1]. The advanced clinical stage prior to surgery and elevated postoperative recurrence rate critically impede the long-term survival prospects for pancreatic cancer patients [2]. Only 20% of patients have the opportunity for pancreatectomy, while 50% of patients have already developed distant metastases [3,4]. Pancreatic cancer is prone to develop resistance to chemotherapy and immune therapy, which limits the long-term survival of cancer patients. In recent years, numerous multi-omics studies have been conducted, which enriches our understanding of pancreatic cancer microenvironment and precancerous lesions. This article aims to provide a comprehensive overview of various aspects of pancreatic cancer, including its epidemiology, etiology, treatment approaches, diagnosis methods, and mechanisms of drug resistance, as well as an exploration of this disease's genomic and clinical features.

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Pancreatic cancer pathogenesis epidemiology, etiology, to mechanism

Epidemiology

In 2018, global statistics indicated 458 918 new instances of pancreatic cancer, resulting in 432 242 fatalities [5]. The majority of pancreatic cancer cases are pancreatic ductal adenocarcinoma (> 90%). The disease manifests slightly more frequently in males than females, with a ratio of 1.4 to 1.0. Metastases often involve organs such as the liver, lymph nodes, lungs, and peritoneum [4]. The low 5-year survival rate of pancreatic cancer is largely

attributed to the advanced stage at diagnosis. Only about 20% of patients present with early and surgically resectable disease, while 50% have metastatic disease and the remaining 30% have locally advanced disease with extensive vascular involvement [2]. Post-surgical patients witness a 5-year survival rate hovering around 15%–25%. Incidences of pancreatic cancer are rare below 40 years of age, yet both its prevalence and mortality rates escalate with advancing age [6–8]. Additionally, the incidence of pancreatic cancer is significantly higher in high-income countries compared to those with a median or low human development index, which is thought to be associated with lifestyle factors [5].

Routine screening for pancreatic cancer in high-risk groups is not recommended as standard practice. Research by Canto and colleagues revealed that around 7% of those deemed at heightened risk for pancreatic cancer received a diagnosis within a 16-year span. The median interval between initial screening and eventual diagnosis stood at 4.8 years (with an interquartile range of 1.6–6.9 years). Prolonged monitoring of individuals susceptible to pancreatic cancer showed that most malignant growths identified during these check-ups were operable, consequently improving the outlook [9]. However, Corral *et al*. demonstrated that in routine endoscopic screening for high-risk individuals, it is estimated that only after screening 135 high-risk patients, can one patient be identified as having high-risk pancreatic lesions [10]. Consequently, additional research is required to identify more efficient screening indicators that can be advantageous for high-risk populations in terms of cancer detection.

Etiology

Short-term factors

Short-term risk factors for pancreatic ductal adenocarcinoma harbor a notably higher degree of peril compared to their long-term counterparts, primarily as they frequently emerge as harbingers of disease progression. Manifestations such as cachexia, the onset of diabetes, and the advent of pancreatitis could all be clandestine indicators of pancreatic cancer. Both weight loss and cachexia could serve as premonitory signals of precancerous stages in pancreatic malignancy, with the ensuing risk escalating in tandem with the degree of weight loss [11]. New-onset diabetes may also suggest the presence of underlying pancreatic cancer. Patients newly diagnosed with diabetes exhibit a 0.3% to 1% likelihood of developing pancreatic cancer within the initial three years following their diabetes confirmation, a rate that notably surpasses that of individuals without diabetes [12–14]. Similarly, pancreatitis may be a consequence of underlying pancreatic cancer, resulting in a significantly higher risk of new-onset pancreatitis within the first year compared to a long-term diagnosis [15].

Long-term factors

Resource from the World Health Organization (WHO) Global Health Observatory database shows that smoking, alcohol drinking, physical inactivity, obesity, diabetes, hypertension, and high cholesterol are long-term risk factors for pancreatic cancer after age-adjustment [16]. Smoking has been identified as the predominant lifestyle risk factor associated with pancreatic cancer. A comprehensive meta-analysis involving 12 case-control studies, with a cohort of 6507 pancreatic cancer patients and 12 890 controls, revealed an odds ratio (OR) of 1.74 (95% confidence interval (CI) 1.61–1.87) demonstrating the correlation between smoking and pancreatic cancer [17,18]. People who smoke more than 35 cigarettes a day have a higher risk of developing pancreatic cancer compared to never-smokers [17]. Cessation of smoking diminishes this risk. Notably, ex-smokers witness a progressive decline in pancreatic cancer risk correlating with the duration since they stopped smoking, yet their risk remains substantially elevated compared to individuals who have never smoked, particularly within the first decade. However, the risk level reverts to that akin to never-smokers after a span of 10–20 years post-quitting [7,18].

Diabetes may be factors that increase the prevalence of pancreatic cancer in younger patients [19]. The long-term risk (> 3 years) of pancreatic cancer increases with the time to diagnosis of diabetes but the short-term risk (< 3 years) decreases as the duration of diabetes diagnosis increases [20,21]. Beyond the mere presence of a diabetes diagnosis, specific diabetes-related biomarkers, such as levels of glucose and insulin, are also linked to an elevated risk of developing pancreatic cancer [7,8].

Obesity [19] and high alcohol intake [22] correlate with a heightened risk ratio, mirroring the extent of their severity. Particularly, excessive intake of alcohol is connected to the onset of pancreatitis, a known precursor for pancreatic cancer [23]. Patients with chronic pancreatitis are at a higher risk of pancreatic cancer owing to persistent inflammation and injury. In a pooled analysis of 14 prospective cohort studies of 862 664 individuals, chronic pancreatitis was associated with a 13.3-fold increased relative risk of pancreatic cancer (95% CI 6.1–28.9) [22].

We found that there is a clear association between the above common long-term risk factors and the demographics of pancreatic cancer. The prevalence of pancreatic cancer in the population was associated with population of smoking, diabetes, obesity and with secondary pancreatitis owing to heavy alcohol

consumption, demonstrating the chances of controlling risk factors to reduce the incidence of pancreatic cancer [6,23].

Environmental factors

Numerous studies delving into the interplay between environmental determinants and the risk of pancreatic cancer, encompassing aspects such as viral infections, occupational exposures, and microbiological constituents, have often yielded incongruent results. Virus infection may be associated with an increased risk of pancreatic cancer. The conclusions about whether hepatitis B and hepatitis C increase the risk of pancreatic cancer are contradictory. Although a meta-analysis of eight studies reported that both hepatitis B and C infection were associated with an increased risk of pancreatic cancer [24], other studies have provided inconsistent conclusions [25–27]. There was no overall association between *Helicobacter pylori* infection and the risk of pancreatic cancer, but some subtypes of *H. pylori* strains may have an impact [28]. Patients are occupationally exposed to aromatic hydrocarbon solvents, pesticides, chlorinated hydrocarbon solvents, formaldehyde, volatile sulfur compounds, and heavy metal exposure with a higher risk of developing pancreatic cancer [29]. Zaitsu *et al*. supported occupational class differences affecting the survival of pancreatic cancer. The service, blue-collar, and unemployed individuals had significantly lower survival rates than white-collar workers [30]. Rare subgroups of oral microbes can also increase pancreatic risk [31]. However, a history of allergy is a protective factor against pancreatic cancer, including a history of hay fever and animal allergy, chronic asthma, and nasal allergy [32].

Inherited factors

Family history of pancreatic cancer

Familial instances of pancreatic cancer may result from a combination of common environmental exposures and inherent genetic predispositions. Individuals with a familial background of pancreatic cancer exhibit a more frequent occurrence of conditions like pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasia (IPMN) within their healthy pancreatic tissue, in contrast to those lacking such family medical history. The risk of pancreatic cancer is also elevated in families with relatives diagnosed with other cancers [4]. However, patients with or without a family history of pancreatic cancer develop the disease in a very similar manner. There were no statistically significant differences in pathological features and somatic mutation profiles between familial and sporadic pancreatic cancer cases [33]. The long-term follow-up results of pancreatic cancer families have also revealed that the risk of developing pancreatic cancer in individuals who do not carry key mutations is close to 0, significantly lower than the 9.3% risk observed in those with pancreatic cancer susceptibility gene mutated (CDKN2A, LKB1/STK11, BRCA1, BRCA2, PALB2, TP53, MLH1, MSH2, MSH6, ATM) [34]. These findings suggest that the genetic basis of familial and sporadic pancreatic cancer might be highly similar.

Genetic susceptibility genes

Multiple genome-wide association studies (GWAS) have pinpointed critically pathogenic germline mutation loci integral to the genesis of pancreatic cancer. Patients harboring germline single nucleotide polymorphisms (SNPs) confront a substantially escalated lifetime risk of pancreatic cancer juxtaposed against their non-mutated counterparts [35]. Germline mutations occur in 4%–10% of pancreatic cancer patients, particularly in those with DNA repair-related mutations, such as homologous recombination defects and Lynch syndrome [36,37]. The BRCA2 pathogenic variant is the most common high-risk genetic variant found in pancreatic cancer patients, accounting for 2%–17% of cases. Individuals with pathogenic BRCA2 variants have a 3.5–5.8-fold increased risk of developing pancreatic cancer compared to those without these variants [38]. Other genes associated with pancreatic cancer risk include PALB2 (< 0.5%), BRCA1 (0.6%–2.2%), ATM (2.3%), STK11 (< 1%), P16/CDKN2A (< 1%–2.5%), PRSS1 (< 1%), MLH1 (< 1%), MSH2 (< 1%), MSH6 (< 1%), PMS2 (< 1%), and various other germline mutations commonly found in syndromes and tumors [39]. Genetic counseling is recommended for both patients and healthy family members who meet certain criteria, such as having a first-degree relative with early-onset pancreatic cancer (< 50 years old), more than one first-degree family member with pancreatic cancer, or a known pathogenic germline gene mutated associated with pancreatic cancer [4,40].

Mechanism

Somatic mutations

The predominant somatic gene alterations in pancreatic ductal carcinoma include KRAS mutations (90%), TP53 mutations (50%–74%). Approximately 90% of all grades of pancreatic cancer have activated oncogenic KRAS mutations. G12D, G12V, and G12C mutations are the most common, while G12R and G12A mutations, as well as other point mutations at codons 11, 13, 61, or 146, appear to be less common [41,42]. KRAS, a membrane-associated GTPase, mediates cellular growth signals via

the MAPK and PI3K pathways. Predominant oncogenic mutations in KRAS are known to disturb the equilibrium of KRAS GTP–GDP cycling [41]. Meanwhile, the deactivation of the tumor suppressor gene TP53 hinders the identification of DNA damage and prevents cell cycle halt, enabling cells to ignore cell cycle checkpoints and resist apoptotic cues. Other mutations are less common, including alternative driver variants in non-KRAS mutant pancreatic cancer, low-frequency mutations associated with precancerous lesions, and critical pathway adaptation mutations.

Remarkably, approximately 10% of pancreatic cancers are devoid of activating KRAS mutations and demonstrate superior outcomes relative to their KRAS-mutated counterparts [41]. These cases are notable for mutations or copy number alterations in alternative drivers such as activating mutations or amplifications of BRAF, FGFR1, or ERBB2, inactivating mutations in NF1, DUSP6, or SPRED1 [43,44] or fusions involving NRG1 and NTRK1 [45–47], alternative oncogenic events genes in SWI/SNF and COMPASS complexes [48], and other familial syndrome genes [43]. Mutations in GNAS are only observed in IPMN with only about 4% of pancreatic cancer patients because IPMN is not a major evolutionary precursor in pancreatic cancer [49]. Most of these low-frequency mutations occur in genes related to cellular processes such as cell survival, cell fate determination, and genomic maintenance [43].

Chromosome alterations

Pancreatic cancer develops complex copy number variants (CNVs) throughout the genome during progression, which are categorized as arm-level and focal-level CNV. Common arm-level alterations include amplifications of 1q (33%) and deletions of 6p (41%), 6q (51%), 8p (28%), 9p (48%), 17p (64%), 17q (31%), 18p (32%), and 18q (71%), which result in significant expression changes in genes, proteins, and phosphoproteins [50,51]. Repeated focal CNVs encompass recognized oncogenic propellants, featuring amplifications in GATA6 (18q11.2), ERBB2 (17q12), KRAS (12p12.1), AKT2 (19q13), and MYC (8q24.2), in addition to deletions in CDKN2A (9p21.3), SMAD4 (18q21.2), ARID1A (1p36.11), and PTEN (10q23.31) [43,52]. Deletions of CDKN2A and SMAD4 are most commonly observed alternations. Alterations in CDKN2A are also early events, while alterations of SMAD4 are late events. Approximately 31%–38% of pancreatic cancer has SMAD4 mutations reducing SMAD4-dependent TGF-β inhibition and promotes non-canonical TGF-β signaling, thereby resulting in reduced cell cycle arrest and apoptosis, promotion of epithelial-mesenchymal transition (EMT) and angiogenesis, and induction of immune suppression, all of which contribute to the progression and metastasis of cancer cells [3,53,54]. Nonetheless, research employing genetically modified mice indicates that a pancreatic-specific lack of SMAD4 does not trigger PanIN or invasive pancreatic cancer [48]. Alterations in the copy number of GATA6 and MYC partially characterize the pancreatic cancer phenotype [48]. Severe imbalance of KRAS and tetraploidization is also more often observed CNV in clinically advanced or metastatic samples, which are critical for molecular subtyping [55].

A key factor in the onset and development of pancreatic ductal adenocarcinoma involves the epigenetic changes impacting oncogenes and tumor suppressor genes. Such alterations, which are reversible, transform the structure of chromatin and histones, thereby modifying the accessibility of gene promoters and the patterns of gene expression. In the context of pancreatic tumor development, histone methylation and acetylation stand out as the most crucial forms of histone adjustments [56]. DNA methyltransferase, including DNMT1, DNMT3A, and DNMT3B, have been shown to be increased in pancreatic cancer and are associated with lower overall survival [57,58]. Methylation of DNA in the promoter regions of tumor suppressor genes like APC, BRCA1, and CDKN2A impedes their transcriptional activities. This phenomenon is considered to be linked to the pathogenesis of human pancreatic cancers [56].

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Spatial transcriptomics

Spatial omics is a well-established concept, with RNAscope and multiplex immunohistochemistry (mIHC) being capable of achieving single-cell resolution for a long time. However, the high cost has limited the widespread use of high throughput methods. In recent years, there has been a gradual increase in the throughput of spatial technologies. Currently, high-throughput spatial technologies can be broadly classified into fluorescence *in situ* hybridization (FISH)-based spatial transcriptomics, sequencing-based spatial transcriptomics, multiplexed imaging based-proteomics, and spatial mass spectrometry-based proteomics. The clinical and translational values of spatial omics in pancreatic cancer have been well elucidated (Table 1) [59]. Current spatial transcriptomics techniques are limited by their throughput and resolution, because the gene expression detected by spatial spots is a mixture [60]. Spatial proteomics has also been used for pancreatic cancer; however, the number of detected genes

Table 1 Summary of select high-throughput spatial transcriptomics of pancreatic ductal adenocarcinoma/ precancerous lesion

Disease	Source	Technologies	Title	Type	Publication date
Pancreatic cancer	Moncada	Spatial transcriptomics team	Integrating microarray-based spatial transcriptomics and single-cell RNA-seq reveals tissue architecture in pancreatic ductal adenocarcinomas	RNA	2020
Pancreatic cancer mice	Sun	10x Visium	Hypoxic microenvironment induced spatial transcriptome changes in pancreatic cancer	RNA	2021
Pancreatic cancer	Zhou	10x Visium	Spatially restricted drivers and transitional cell populations cooperate with the microenvironment in untreated and chemo-resistant pancreatic cancer	RNA	2022
Pancreatic cancer	Hwang	DSP	Single-nucleus and spatial transcriptome profiling of pancreatic cancer identifies multicellular dynamics associated with neoadjuvant treatment	RNA	2022
Pancreatic cancer	Barkley	10x Visium	Cancer cell states recur across tumor types and form specific interactions with the tumor microenvironment	RNA	2022
IPMN	Sans	10x Visium	Spatial transcriptomics of intraductal papillary mucinous neoplasms of the pancreas identifies NKX6-2 expression as a driver of gastric differentiation and indolent biological potential	RNA	biorxiv
IPMN	Agostini	10x Visium, GeoMx	Transcriptomic dissection of intraepithelial papillary mucinous neoplasms progression by spatial technologies identified novel markers of pancreatic carcinogenesis	RNA	biorxiv
PanIN	Bell	10x Visium	PanIN and CAF transitions in pancreatic carcinogenesis revealed with spatial data integration	RNA	biorxiv

Abbreviations: IPMN, intraductal papillary mucinous neoplasia; PanIN, pancreatic intraepithelial neoplasia; CAF, cancer-associated fibroblast; NKX6-2, NK6 homeobox 2.

has been limited (< 50) compared to spatial transcriptomics [61,62].

Moncada *et al*. published the first protocol using the Multimodal Intersection Analysis (MIA) algorithm for single-cell sequencing and spatial transcriptomics mapping. They found that macrophages were most enriched immune cell type in pancreatic cancer. Moreover, specific subgroups of ductal cells, macrophages, dendritic cells (DCs), and various other cell types show a spatially confined enrichment in certain compartments. Particularly, M1 macrophages are predominantly found in stromal areas as well as cancerous tissues, indicating the presence of an inflammatory milieu in these zones [63]. Hwang *et al*. [64] employed NanoString GeoMx DSP to unravel the spatial arrangement of cells and expression patterns within multicellular structures. Their findings highlighted that the majority of malignant programs demonstrated greater variability between individual patient tumors than among different regions of interest (ROIs) within a single tumor. However, mesenchymal, immunomodulatory, and myofibroblastic progenitor programs were relatively more stable. Notably, the neural-like progenitor and neuroendocrine-like malignant programs were more prevalent in ROIs derived from tumors treated with chemoradiotherapy (CRT) compared to those untreated, aligning with insights from single-nucleus RNA sequencing (snRNA-seq) analyses. These spatial relationships encompass both extensive multicellular communities, evident from clustering analysis, and more nuanced features seen in specific association pairs. Zhou *et al*. [65] used a spatial sequencing platform to find the spatial distribution of pancreatic cancer with different driver mutations status. Validating acinar-to-ductal metaplasia (ADM) using spatial transcriptomics data remains challenging due to the lack of single-cell resolution and the scarcity of ADM cells. Nonetheless, regions containing ADM cells might exhibit expression markers characteristic of both acinar and ductal cells, a phenomenon that aligns with the findings presented by Tosti *et al*. [66]. Zhou *et al*. identified NECTIN4 as the NECTIN most specifically associated with tumor cells. Through spatial transcriptomics data, our analysis concentrated on two particular regions to examine the expression of TIGIT in areas close to regions infiltrated by lymphocytes. This investigation revealed a colocalization of tumor regions in the H&E stains with NECTIN4 expression, underscoring the potential of the TIGIT–NECTIN4 axis as a therapeutic target to enhance anti-tumor T cell activity [65]. Barkley *et al*. [67] utilized spatial data to demonstrate the induction of a squamous module in pancreatic cancer, pointing to a process of squamous differentiation. This includes differentiating between classical subtypes (characterized by elevated expression of acinar-ductal genes like TFF1 and CEACAM6) and basal subtypes (marked by higher levels of squamous and basal genes, such as LY6D and KRT15). The heightened expression of squamous markers in pancreatic cancer implies a frequent partial metaplasia, steering toward a squamous program [68]. Progressive pancreatic cancer expresses more squamous-associated genes compared to normal pancreas, and

squamous staging has been associated with poorer prognosis. It is important to note that changes in gene expression do not imply pathomorphologic conversion of adenocarcinoma to squamous or adenosquamous carcinoma, which are rare in histology [69]. Spatial results were also used to visualize hypoxic microenvironment-induced spatial transcriptome changes in pancreatic cancer [70]. Bell *et al*. applied migration learning to integrated PanIN spatial transcriptomics with single-cell RNA sequencing (scRNA-seq) data, allowing analysis of cellular and molecular progression from PanIN to pancreatic cancer. Using PanIN samples, it was found that PanIN lesions are predominant in the classical pancreatic cancer subtype. For the first time, it was observed that the same cancer-associated fibroblast (CAF) subtype (myofibroblasts (myCAF), inflammatory CAF (iCAF), and antigen-presenting CAF (apCAF)) present in aggressive pancreatic cancer is also present in human precancerous lesions. CAF-PanIN interaction promotes inflammatory signaling in tumor cells. As PanIN progresses to pancreatic cancer, tumor cells shift to proliferative signaling [71]. Two other unpublished studies identified NKX6-2 as a key transcription factor for the fatal transition in IPMN using spatial transcriptomics [72,73].

Single cell screening

In the past, we used bulk RNA sequencing (RNA-seq) results using paired tumor and normal specimens, and the sequencing results are mixture of each cell. The single cell era has greatly enriched our understanding of tumor microenvironment (TME) of pancreatic cancer, which provides us further insights of tumor and TME cells.

Untreated pancreatic cancer cells

Peng *et al*. [74] classified untreated pancreatic cancer separated into type 1 and 2 ductal cells using single cell RNA sequencing results. Type 1 ductal cells are relative normal and present in both non-cancerous and neoplastic tissues, which were enriched for cell adhesion, migration, and inflammatory response, while type 2 ductal cells were enriched for malignant phenotypes such as proliferation and hypoxia. Repair of the damaged pancreas generates ADM, which is essential for recovery. Although this repair process is reversible, post-repair dedifferentiation can be prevented by the introduction of KRAS mutations and recurrent inflammation, which leads to the formation of PanIN, a precursor condition for pancreatic cancer. In addition, concomitant deletion of tumor suppressors such as p53 can directly lead to pancreatic cancer initiation, and tuft cells can also convert to PanIN after mutation [75].

Surgically resected primary tumor is different from sampling results. Because metastatic puncture samples from recurrent metastases have a higher proportion of epithelial cells compared to recent scRNA-seq studies of surgically resected pancreatic cancer tumors, the most common immune cell types are myeloid and T cells, both monocytes and the higher proportion of invasive basal-like cell subtypes found in metastases by bulk RNA-seq are more likely to be present [49,76,77].

Fibroblasts

Fibroblasts are key cells in the mesenchymal environment of pancreatic cancer, and fibroblasts have pro-tumor proliferation and EMT effects [78]. Both iCAF and myCAF can be conservatively identified in most cancers. iCAF is characterized by $ACTA2^{low}$ FAP^{high} and myCAF is characterized by $ACTA2^{high}$ and most of the single cell studies require that fibroblasts in pancreatic cancer have ACTA2 expression. These two fibroblast populations are the most common fibroblast subpopulations in all prior tumors and can be conservatively identified in multiple single-cell sequencing. Most of the cell subpopulations in single-cell studies of pancreatic cancer can be classified into these two groups. apCAF is further suggested by the three CAF isoforms (myCAF, iCAF, and apCAF) described by Elyada *et al*. apCAF subpopulation is defined as $CD74^{+}HLA^{+}CD45^{-}$ fibroblasts, however, this idea remains clearly controversial [79]. apCAF is sometimes difficult to be conservatively identified. In particular, apCAF has a very low percentage by itself and $HLA^{+}CD74^{+}$ is widely expressed between cells. The CAF derived from murine pancreatic cancer forms a separate cluster, whereas apCAF detected in human pancreatic cancer is scattered in the iCAF and apCAF clusters. A number of pancreatic cancer single-cell data set analyses have failed to identify apCAF, although they do not deny this possibility. One thing is confirmed, apparently in human pancreatic cancer single-cell data sets, apCAF is not able to form an independent group like iCAF and myCAF [64,76]. Another paper by Zhou *et al*. not only identified apCAF, myCAF, iCAF, but also $CD133^{high}$ iCAF and $CXCR4^{high}$ iCAF, $CD133^{high}$ iCAF expressing both stem cells and the epithelial marker EPCAM, and $CXCR4^{high}$ iCAF expressing strongly CD45. These two categories are like epithelial and immune cells, and we still need to look at them with caution [65]. Fibroblast cells are conserved across organs. Since there is no gold standard for fibroblasts, there are a variety of typologies, such as steady-state-like (SSL), mechanoresponsive (MR), and immunomodulatory (IM) CAFs [80]. scRNA-seq reveals stromal evolution into $LRRC15^{+}$ myCAF as a determinant of patient response to cancer immunotherapy. Dominguez found $PDPN^{+}$ cells are the dominant fibroblast population in normal and pancreatic cancer. Levels of the $LRRC15^{+}$ CAF were correlated with poor

response to anti-PD-L1 therapy. Late-stage tumors support previous observations identifying IL-1-driven iCAF and TGF-β-driven myCAF [81]. Such results explain the complex crosstalk between fibroblast heterogeneity and cancer immunity [82–84].

Immune cells

Steele *et al*. compare the difference of immune cells from blood to pancreatic tissue [85]. Multiomic profiling of lung and liver tumor microenvironments of metastatic pancreatic cancer reveals site-specific immune regulatory pathways, TME of lung generally exhibits higher levels of immune infiltration, immune activation, and pro-immune signaling pathways in lung cancer, whereas multiple immune-suppressive pathways are emphasized in the liver TME [86]. Within the myeloid cluster, subclustering revealed six distinct populations that were identified as resident macrophages, alternatively activated M2-like macrophages, classic monocytes, cDC1, and two types of Langerhans-like DC. Within T cell cluster, subclustering identified four discrete cell types: $CD8^+$ T cells, $CD4^+$ T cells, regulatory T cells (Tregs), proliferating T cells. In addition, several groups have examined features of the tumor immune microenvironment during the tumorigenic process, but suitable immune checkpoint blockade therapy in pancreatic cancer is still lacking [87].

Treated pancreatic cancer

scRNA-seq reveals the effects of chemotherapy on human pancreatic adenocarcinoma and its tumor microenvironment. Different iCAF were observed under different chemotherapy analyses. Naïve patients are both low in two lists of genes, with HSPA1A1, HSPA1AB, DNAJB1, FOS, JUN, FOSB high after FOLFIRINOX (5-FU/calcium folic plus oxaliplatin and irinotecan), and MT1X, MT1M, MT1E, and MT2A high after gemcitabine (GEM) plus nab-paclitaxel [65]. Hwang and his colleagues, Shiau *et al*., presented a single-cell resolution framework of diverse remodel of states and distributions in endothelial cells enriched with vasculogenesis, stem-like state, response to wounding and hypoxia, and endothelial-to-mesenchymal transition (reactive EndMT). A bulk transcriptome analysis of two independent cohorts (n = 269 patients) revealed that the lymphatic and reactive EndMT lineage programs were significantly associated with poor clinical outcomes [88].

High-throughput sequencing application

Other high-throughput sequencing technologies are also widely used in the study of pancreatic cancer, including high-throughput/resolution chromosome conformation capture (HI-C) sequencing technology, the third generation DNA/RNA sequencing technology, cleavage under targets and release using nuclease (CUT&RUN), methylation sequencing technology, epigenomics, and other technologies. Although these technologies are characterized by having very good sequencing results of cancer cell lines, results in pancreatic tissues need to be interpreted with caution, due to the low tumor purity of pancreatic cancer.

(1) Long-read sequencing: Du *et al*. discovered structural variants as well as chromatin structure of pancreatic cancer using Hi-C and long-read sequencing. Structural variants are defined as variants more than 50 bp, and structural variants located in key genetic regions can also drive tumor malignant progression [89].

(2) scATAC-seq: Single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq) and single-cell combinatorial indexing ATAC-seq (sci-ATAC-seq) are effective tools in the study of genome-wide chromatin accessibility landscapes. Chromatin region accessibility play essential roles in epigenetic regulation have been measured at the single-cell level using single-cell ATAC-seq approaches [90,91].

(3) scCOOL-seq: scCOOL-seq is a single-cell multi-omics sequencing technology that can analyze DNA methylation and chromatin accessibility togather. Fan *et al*. found ZNF667 and ZNF667-AS1, whose expressions were linked to a better prognosis for pancreatic cancer patients by affecting the proliferation of cancer cells and revealed the critical epigenomic features of cancer cells in pancreatic cancer patients at the single-cell level [92,93].

Molecular subtypes

Waddell *et al*. used the results of high-throughput DNA sequencing to establish the first molecular typing of pancreatic cancer based on genomic variants, and classified pancreatic cancer into stable, locally rearranged, scattered and unstable [94]. The molecular typing of pancreatic cancer was established for the first time based on genetic and genomic variant sequencing. In 2011, Collisson *et al*. reported three molecular subtypes of pancreatic cancer based on the analysis of 27 microdissected pancreatic cancer samples: classical, quasimesenchymal (QM-PDA), and exocrine-like. In 2015, Moffitt *et al*. [95] clustered two states of pancreatic ductal adenocarcinoma epithelium into 2 types: classical and basal. The classical types of high GATA6 expression and the basal subtypes have stronger EMT, and the basal types have more squamous cell component expression, and worse prognoses. In 2016, Bailey *et al*. [69] used unsupervised clustering of RNA-seq data from 96 tumors with at least 40% epithelial content and yielded four subtypes: squamous, pancreatic progenitor, immunogenic,

and abnormally differentiated endocrine exocrine (ADEX). Raphael *et al*. [43] found that reconciliation of previously identified subtypes and high-purity tumors can consistently be classified into either the basal-like/squamous or the classical/progenitor subtype. Although no entirely new typing was introduced, it was suggested that the results of three major methods of molecular classifications were heavily influenced by tumor purity, and that high-purity samples conformed to the classical-basal class, establishing the Moffit typing in pancreatic ductal cell carcinoma. In 2018, Puleo *et al*. [96] found the exocrine-like (Collisson *et al*. [97]) and ADEX (Bailey *et al*.) subtypes resulting from a mixture of pancreatic endocrine cells, acinar cell contamination, or acinar cell carcinoma, and refined the subtypes into activated, desmoplastic, pure classical, and immune subtypes. Given that Moffit has become the most important subtype reflecting pancreatic ductal cell carcinoma, it is limited by the software calculations responsible and inconsistencies in tumor purity. Significant differences appeared in the tissue composition of pancreatic tumors in the Moffitt, Bailey, and TCGA cohorts. Up to 45% of tumor samples from various public cohorts were reclassified after removal of non-epithelial signals from tumor bulk expression profiles [98]. Indeed, there was significant sampling heterogeneity across tumor sites and it is inconclusive whether this sampling error affects the correct therapeutic staging of patients [99]. In 2020, Rashid *et al*. create the benchmark classifier Purity Independent Subtyping of Tumors (PurIST) to allow clinicians to better reproduce Moffitt subtypes for all samples in clinical trials in bulk RNA-seq [100]. PurIST found a purity-independent subtypes of classical and basal-like subtypes, using the NanoString platform and the Illumina sequencing platforms for bulk RNA-seq results. Moffitt typing of pancreatic cancer RNA-seq results on different platforms can be performed with over 95% accuracy based on a simple formula without any fiber-cutting techniques, however, unfortunately this tool cannot be used for single cell sequencing results. In 2022, CPTAC–pancreatic ductal adenocarcinoma (PDAC) started the first addition of proteomics multimodal typing, using multi-omics NMF typing to classify pancreatic cancer into 4 subtypes, and found that the typing is highly influenced by sampling location and purity. When retaining high purity samples of pancreatic cancer, the samples can be two classes, the typing with Moffitt dichotomy including Cluster 1 (Moffitt classical) and Cluster 2 (Moffitt basal), which is highly correlated with prognosis. It is important to note that protein-based dichotomization or RNA dichotomization is not always consistent. xCELL typing was used by CPTAC–PDAC to determine immune hot-cold subtypes and found that 92.3% samples (108 samples/117 samples) had little or no immune infiltration. Only 7.7% of the samples were from immune hot tumors, and immune hot tumors had significantly lower tumor purity. If 40 samples rich in follicular and islet cells were removed (probably due to essential tumor characteristics or early tumor staging or insufficient tumor composition at the location of harvest), 88.5% (69 samples/78 samples) patients were definite immune cold tumors. 11.5% of the visualized sections were examined after removing false positives at the sampling site due to mixed lymph nodes. The remaining 88.5% (69 samples/78 samples) had definite immune cold tumors [50]. These findings support that pancreatic cancer is a naturally immunotherapy-resistant tumor with little clinical response to PD-1/PD-L1 blockade therapy. The concomitant endothelial cell remodeling characterized by decreased expression of endothelial adhesion proteins, accompanied by elevated VEGF and hypoxic pathways, increased glycolysis and dysregulation of cellular junctions in immune-cold pancreatic cancer may form a common inhibitor of immune cell infiltration. Adapting pancreatic cancer to immune hot tumors will be the most important target in the future [101–103]. The most important contribution of CPTAC–PDAC is the validation limiting the previous staging of immune prototype tumors to a very small fraction of pancreatic cancers and the need to exclude false positives.

In 2020, COMPASS/PanCuRx began to explore molecular subtypes using both bulk RNA-seq and scRNA-seq, and they classified pancreatic cancer into 5 groups: basal-like A, basal-like B, hybrid, classical A, classical B [55]. Similar to the coexistence of basal and classical programs within the tumor, this mixture of cell states may be more consistent with the true response of tumor cell heterogeneity. Patients with basal-like A pancreatic cancer usually present with advanced disease and have the worst response to (GEM)-based chemotherapy and FOLFIRINOX. In contrast, patients with basal-like B and mixed tumors usually present with resectable disease. Thus, the ability to distinguish basal-like A, basal-like B, and hybrid subtypes from groups previously classified as basal-like allows for more accurate prediction of chemotherapy response. Classical A/B tumors were found to be associated with increased frequency of GATA6 amplification and complete loss of SMAD4, whereas basal-like A/B tumors showed complete loss of CDKN2A and higher frequency of TP53 mutations. At single-cell resolution, results of COMPASS/PanCuRx also show that basal-like and classical subtypes can coexist in the same tumor, highlighting the molecular heterogeneity within the tumor. In fact, many subtypes are not absolutely mutually exclusive and it is more like determining which subtype is characterized by a dominant feature in the overall development as the subtype to which the patient belongs [104]. The patient's

strain is determined by the characteristics of the patient. A change in the dominant cell population may occur after patients underwent pradiation, chemotherapy, or immunotherapy. The use of FOLFIRINOX or GEM plus nab-paclitaxel presented different expression profiles of pancreatic cancer [65]. In the era of single cell, in addition to emphasizing the qualities of the cells themselves, the concept of cell state began to be introduced, where changing the surrounding environment differently exerts a completely different effect on the tumor cells, an effect that can be traced back to specific cell types [64,105]. Raghavan *et al*. stated that the EMT program is positively correlated with basal-like features and negatively correlated with classical features possessing more basal-like typing in metastatic samples. This may be related to the copy number imbalance of KRAS. Single cell-based typing classified the pancreatic cancer into scBasal, intermediate co-expressor (IC), and scClassical subtypes, using single cell results [105]. An updated molecular subclassification of pancreatic cancer was reported by Hwang *et al*. in 2022. Using snRNA-seq and spatial transcriptome analysis of 43 major pancreatic cancer samples (18 untreated and 25 treated), they identified three distinct subtypes: classical, squamous-basal, and treatment-enriched. Their study found that the NRP malignant cell program is enriched for residual cancer after radiotherapy. NRP cells are associated with pancreatic cancer through regulation of drug efflux-related genes, negative regulation of cell death, chemoresistance (e.g., ABCB1, BCL2, PDGFD, and SPP1), tumor-neural crosstalk (e.g. SEMA3E, RELN, and SEMA5A) and metastasis (NFIB) of patients with treatment resistance and low survival rates. We identified 14 malignant cell programs that reflected either lineage (classical, squamoid, basaloid, mesenchymal, acinar-like, neuroendocrine-like, and NRP) or cell state (cycling-S, cycling-G2/M, MYC, interferon, tumor necrosis factor/nuclear factor κB (TNF-NFκB), ribosomal and adhesive pathways) and four CAF programs (myofibroblastic progenitor, neurotropic, immunomodulatory, and adhesive). These molecular classifications of pancreatic cancer provide a rich and comprehensive data set to better understand pancreatic tumorigenesis, genetic/molecular landscape, intra- and inter-tumor heterogeneity, tumor progression, and drug resistance. More importantly, molecular subtyping of pancreatic cancer may provide useful information for more effective subtype-tailored treatment of pancreatic cancer patients. However, due to their complexity, these classifications of pancreatic cancer have not yet been used in routine pathological diagnosis or clinical practice (Table 2).

ADM

ADM is thought to be the primary origin of pancreatic precancerous lesions that eventually develop into pancreatic ductal adenocarcinoma. The origin of pancreatic ductal adenocarcinoma includes: (1) conversion of acinar cells to PanIN via ductalization and eventually to pancreatic cancer; (2) conversion of acinar cells to other receptor cell types such as tuft cells, and mutation of susceptible cells to malignant cells; (3) direct carcinogenesis of ductal cells; (4) abnormal transformation of pancreatic progenitor cells into tumors; (5) formation of pancreatic cancer through other precancerous lesions secondary to cancer. The ADM origin theory is the most mainstream theory because it can explain the sharp decrease in the number of normal cellular follicular cells, endocrine cells in pancreatic cancer tissue sections. Acinar cells are highly plastic and have the potential to

Table 2 Molecular subtypes of pancreatic ductal adenocarcinoma

Study	Techniques	Subtypes	New subtype
Waddell	WGS	Stable, locally rearranged, scattered and unstable	Yes
Moffitt	Bulk RNA	Classical, basal-like	Yes
Rashid	Bulk RNA	No new; PurIST classifers for classical, basal-like	No
Collisson	Bulk RNA	Classical, quasi-mesenchymal, and exocrine-like	Yes
Bailey	Bulk RNA	Squamous, pancreatic progenitor, immunogenic, ADEX	Yes
Puleo	Bulk RNA	Pure basal like, stroma activated, desmoplastic, pure classical, and immune classical	Yes
Chan-Seng-Yue	Bulk RNA	Basal-like A, basal-like B, hybrid, classical A, classical B	Yes
Raphael	Multiomics	No new; high purity samples into basal-like/squamous or classical/progenitor	No
Cao	Multiomics	All into 4 subtypes; high purity into basal-like or classical	No
Hwang	Single cell RNA-seq	7 malignant lineages: NRP, SQM, MES, ACN, NEN, BSL, CLS, cell state (cycling-S, cycling-G2/M, MYC, interferon, TNF-NFκB, ribosomal and adhesive pathways)	Yes
Raghavan	Single cell RNA-seq	scBasal, IC, and scClassical	Yes

Abbreviations: WGS, whole genome sequencing; ADEX, abnormally differentiated endocrine exocrine; IC, intermediate co-expressor; NRP, neural-like progenitor; SQM, squamoid; MES, mesenchymal; ACN, acinar-like; NEN, neuroendocrine-like; BSL, basaloid; CLS, classical; TNF-NFκB, tumor necrosis factor/nuclear factor κB.

transform into ductal epithelium or endocrine epithelium [66,106]. ADM may be a decisive step in the tumorigenic process, selecting plastic cells for more aggressive subsequent tumorigenesis by transforming acinar cells into malignant cells and precursor fine cells. The formation of ADM was found to be regulated by two major signaling pathways downstream of KRAS (PI3K/Akt and MEK/Erk). Among them, RAC1 is an important target downstream of PI3K/Akt signaling that mediates actin polymerization to redistribute filamentous actin from the apical to the basal part of the acinar cell, leading to apico-basal mechanical tension imbalance. The ADM process in KRAS mutant mice is irreversible and progresses to PanIN, which eventually develops into pancreatic cancer. The pancreatic intraepithelial neoplasia is a unique inflammatory microenvironment-induced pathway of acinar–ADM/PanIN–pancreatic cancer evolution [107]. Two published landscape of combined single-cell and spatial-omics analyses for pancreatic cancer have established temporal model based on the acinar–ADM–PanIN/atypical duct–pancreatic cancer hypothesis [64,65]. Although the progressive model occupies residency, the question of the sequence of ADM and PanIN or whether they must undergo the abovementioned progressive process is not conclusive [108,109].

ADM is defined by conversion of pancreatic acinar cells to abnormal ductal epithelial adenocarcinoma. Using a mouse model where activation of KRAS G12D and deletion of Fbw7 or p53 in acinar cells leads to ADM, Krt19-positive globular lesions with a continuous ductal tree can be observed [107]. It has also been theorized that ADM is a physiological, rapid, and reversible adaptive response capable of limiting the adverse effects of persistent stimuli such as repetitive pancreatitis, with rapid dedifferentiation of acinar cells, thereby producing an effective response by rapidly reducing acinar zymogens (such as amylase, elastase, and pancreatic ribonuclease) to effectively limit tissue damage and enable rapid and complete recovery from it. This adaptation is able to rapidly reactivate ADM during subsequent inflammatory events. Some ADM cells acquire KRAS mutations during this process to adapt to the environment [110].

In contrast to tumor cells, the ADM population expresses oncogenes and significantly upregulates epithelial-mesenchymal transition and stem cell genes. The unique expression pattern of ADM as an intermediate state suggests a dynamic shift between tumor and follicular fate and a role in initiating pancreatic tumorigenesis by gaining access to drive the progression of KRAS events to pancreatic cancer [111]. Single cells make it possible to identify the transition state ADM, which Zhou *et al*. refer to as ADM_Normal for expression similar to that between the follicular cells and the normal ductal lineage, and ADM_Tumor for transition cells between the follicle and PanIN. While both ADM_Tumor and ADM_Normal showed a decline in the expression of follicular markers, they conversely exhibited an uptick in the expression of pancreatic cancer indicators and catheter-associated markers, respectively. A small fraction of ADM_Tumor cells displayed this trend, indicating that ADM_Normal may represent a transitional phase more akin to regular ductal cells, largely devoid of genomic changes. In contrast, ADM_Tumor appears to be more comparable to PanIN, carrying certain genomic modifications (for instance, CDKN2A, aneuploidy) [65]. Hwang *et al*. included low CNA cells within the epithelial compartment that co-expressed ductal and follicular lineage markers, and these cell subpopulations were defined as transitional state cells (ADM and non-classical ductal cells). An inferred pseudotemporal trajectory from acinar to ADM to atypical ductal to malignant cells was inferred using the inferred pseudotemporal trajectory and found to parallel the monotonic increase in positive regulation of KRAS signaling, supporting ADM and atypical ductal cells as relevant intermediate states in pancreatic cancer tumorigenesis. Hwang *et al*. identified a set of low CNA nuclei within the epithelial compartment in the presence of co-expressed ductal and follicular lineage markers, which may reflect ADM. In addition, a distinct subpopulation of ductal cells express high levels of ductal (e.g., CFTR) and malignant (e.g., KRT5 and KRT19) markers without elevated CNA, which we termed atypical ductal cell expression, if also expressing HG PanIN signature genes (e.g., KRT17) is represented, then ADM converts to an atypical ductal state.

Another part argues that conversion from ADM is also required to undergo chemotaxis of receptor cells, which subsequently mutate before entering the oncogenic process. For example, partial conversion of adenoidal blast cells to $HNF1B^+$ or $POU2F3^+$ ADM populations leads to neoplastic transformation and formation of $MUC5AC^+$ gastric-pit-like cells, including EEC, tufts cells, etc. Subsequent KRAS mutations activate cell differentiation to cancer epithelium [66,75]. DCLK1-positive cells represent a reservoir of pancreatic progenitor cells, which can maintain epithelial and *in vivo* follicular regeneration after tissue damage, and these cells are prone to develop into pancreatic cancer [110].

It is important to note in particular that although single cells allow us to gain insight into many transitional state cells and precancerous lesions of pancreas, we need to view these results with caution. Although many of the single-cell sequencing studies investigating ADM in recent years have claimed to use algorithms or manual single-cell removal of apparent doublets, coupled with

spatial validation, there is still a significant false-positive rate. Because of similar ductal-acinar adhesion, cell in cell [112,113] can also present false ADM lesions, these effects cannot be fully removed by single-cell dissociation techniques and algorithms [114,115]. Therefore, based on the fact that pancreatic cancer single cells in particular can present false ADM lesions, the results of ADM analysis based on single cells of pancreatic cancer in particular still need to be clarified by subsequent experiments.

High risks precursor diseases: inflammation, precursor lesions and neoplasms

Chronic pancreatitis

Chronic pancreatitis is a disease caused by progressive inflammation and irreversible fibrosis caused by cumulative damage to pancreas over time. Excessive extracellular matrix deposition eventually leads to failure of internal and external hormone secretion. It usually presents with recurrent episodes of abdominal pain or pancreatitis and can progress to pancreatic cancer. A large subgroup of patients with neither pain (nearly 30%) nor a previous diagnosis of acute pancreatitis (about 50%) were also classified as having chronic pancreatitis. Chronic pancreatitis is usually associated with alcohol consumption, smoking or genetic risk factors, which notably increases the risk of pancreatic cancer. Sporadic chronic pancreatitis patients have a cumulative risk of 1.8% and 4% at 10 and 20 years, respectively, while those with hereditary pancreatitis have a 7.2% risk by age 70 years [116,117]. The annual incidence is 5 to 8 per 100 000 adults with a prevalence of 42 to 73 per 100 000 [116]. Common genetic mutations linked to chronic pancreatitis include CFTR, SPINK1, and CTRC, with over 90% developing early-onset pancreatic cancer [118]. Mouse models edited for genes like Spink1, Prss3b, and Cpa1 mirror the progression seen in humans with germline mutations [119–121]. Hereditary pancreatitis, accounting for about 1% of all cases [116], is an autosomal dominant condition resulting from PRSS1 gene mutations. Despite its origin, chronic pancreatitis invariably increases pancreatic cancer risk. No consensus exists for cancer surveillance, but experts underline the importance of thorough monitoring, especially if symptoms like unexplained weight loss or new-onset diabetes are observed [122].

Chronic pancreatitis can cause increased susceptibility to pancreatic cancer by inducing ADM or by generating susceptible precursor cells. Preneoplastic pancreatic alterations have been previously identified in acute and chronic pancreatitis [123]. ADM rapidly shut down pancreatic enzyme expression, which may be able to limit the adverse effects of repetitive pancreatitis, allowing pancreatic tissue to recover rapidly from inflammation. However, persistent inflammatory stimulation of chronic pancreatitis results in extensive ADM persistance without recovery, increasing the risk of carcinogenesis. Any genetic event that promotes or stabilizes ADM, such as activating mutations in KRAS, may lead to the eventual progression to pancreatic cancer [110]. Tuft cells, a cancer precursor cell type, were commonly discovered in chronic pancreatitis and pancreatic cancer [65,66]. Also, reprogramming of DCLK1-positive cells may play an important role in inflammation-promoted tumorigenesis [110], which are present in chronic pancreatitis [124–126].

Precursor lesions and neoplasms

The current WHO classification of tumors of the digestive system recognized five types of potential pancreatic cancer precursors: PanIN, mucinous cystic neoplasm (MCN), intraductal oncocytic papillary neoplasm (IOPN), intraductal tubulopapillary neoplasm (ITPN), and IPMN [59]. Fig. 1 shows the common driving mutation genes of precancerous lesions, and the proportion of pancreatic cancer derived from each lesion. Most pancreatic precancerous lesions are usually macroscopic lesions > 0.5 cm, while PanIN is a microscopic lesion < 0.5 cm. In the past, the precancerous lesions were graded into three grades (low, moderate, and high). There was a general lack of consensus diagnostic markers to distinguish low-grade (LG) nonmalignancy from high-grade (HG) malignancy. Those previously classified as LG and “moderate grade” are now classified as LG, and those previously classified as HG remain the same. Many precancerous lesions are found incidentally after surgery. Despite the availability of laser capture microdissection techniques, the study of precancerous lesions in pancreatic cancer is still lacking. Nowadays, the availability of spatial omics allows obtaining microscopically diagnostic-dependent lesions for study using FFPE samples, which has significantly improved the understanding of pancreatic lesions (Table 1).

IPMN

IPMN is defined as an apparently visible (usually > 5 mm), predominantly shaped or rarely flattened, noninvasive, mucin-producing epithelial tumor in the main ducts or branch ducts. The risk factors for the development of IPMN are unclear. The majority of IPMNs are located in the head of pancreas, and 40% of cases are multicentric. Median age of patients with IPMNs with invasive carcinoma is 3–5 years older than that of those without invasive carcinoma, indicating that

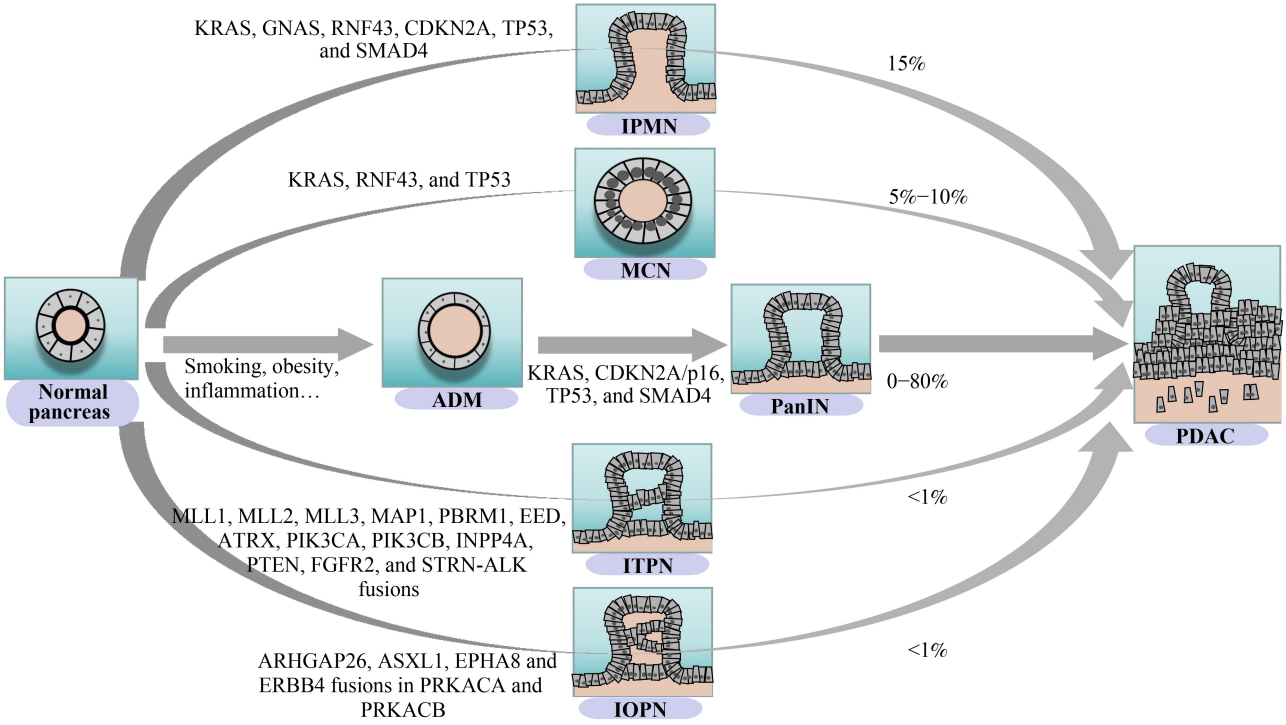

Fig. 1 Patterns and common mutated genes of precursor lesions of pancreatic cancer. Abbrevations: ADM, acinar-to-ductal metaplasia; PanIN, pancreatic intraepithelial neoplasia; MCN, mucinous cystic neoplasm; IPMN, intraductal papillary mucinous neoplasia; IOPN, intraductal oncocytic papillary neoplasm; ITPN, intraductal tubulopapillary neoplasm.

progression to cancer takes time. IPMNs can be classified as “main duct type,” “branch duct type,” and “mixed duct type” according to their relationship with the main pancreatic duct. The main pancreatic duct type IPMN have higher risk of carcinogenesis than the other types. Mural nodules and/or irregular ductal contours may suggest HG neoplasms or invasive carcinoma.

Pathological subtypes of IPMN are divided into gastric type, intestinal type, and pancreatobiliary type. Gastric-type IPMNs are usually LG, intestinal type may be low or HG pancreatobiliary IPMN [127]. Intestinal-type IPMNs exhibit biological variances from gastric or pancreatobiliary types, encompassing disparities in the frequency of driver mutations (detailed in subsequent sections) and in the predominant and branching duct locations. Somatic alterations in hotspots of the KRAS oncogene represent some of the initial genetic shifts in IPMNs, whereas mutations in CDKN2A, TP53, and SMAD4 arise at more advanced stages (refer to Fig. 1). Furthermore, genomic analyses of IPMNs have uncovered additional driver genes uniquely characterized by a high mutation frequency confined to IPMNs. For example, mutations in GNAS are common in IPMN, which are thought to be alternative triggers for KRAS tumor formation. The prevalence of GNAS mutation varies by pathological subtypes, and thus the prevalence of GNAS mutation reported in IPMN cohorts varies, ranging from 40% to 80%. Most intestinal IPMNs are predominantly ductal lesions with a high frequency of GNAS mutations. Furthermore, RNF43, a tumor suppressor gene, frequently exhibits inactivating mutations in IPMNs, believed to surface following initial alterations in KRAS and GNAS. While RNF43 mutations are sparingly seen in pancreatic cancer, their significant occurrence is specific to IPMNs. Another distinctive prospective driver in IPMN development, KLF4, has been pinpointed in association with low-grade (LG) IPMNs. In-depth multi-regional and single-cell sequencing investigations into IPMNs have uncovered considerable genetic diversity in driver mutations. Interestingly, LG IPMNs often harbor several distinct KRAS or GNAS mutations present in separate cells, delineating various clones devoid of other somatic mutations. This implies a potential polyclonal origin for many IPMNs. PanIN and gastric IPMN are distinguished based on morphology and size and show the same immunohistochemical profile, with diffuse positivity for MUC5AC, without MUC1 and MUC2 expression. Intestinal IPMN are distinctly different lesions at both morphological and immunohistochemical levels and are characterized by positive MUC2 and MUC5AC [128]. A previous study found that in approximately 20% of cases, concurrent IPMN and adenocarcinoma were independent of each other, and IPMN proved to be a mere parallel event in a subset of pancreatic cancers [129,130]. The study found that IPMN and adenocarcinoma were independent of each other in

about 20% of cases, and IPMN proved to be a simple parallel event in a proportion of pancreatic cancers. The unique epigenetic control and expression patterns of MUCL3, coupled with frequent copy number variations (primarily deletions) in gastric IPMNs, could signal a heightened propensity for these lesions to progress. Intestinal IPMNs, characterized by a distinct genetic composition and elevated genomic instability, manifest higher proliferation rates even in lower-grade lesions, indicating a potentially greater risk of progression compared to PanINs and gastric IPMNs. Unlike PanINs and gastric IPMNs, intestinal IPMNs exhibit an increased presence of genes related to mucin secretion and distinctly divergent epigenetic landscapes informed by DNA methylation patterns, which correlate them with various mature cell types in the ductal array. Histopathological subtyping employs markers like MUC1, MUC2, MUC5AC, and CDX2. The spatial transcriptome of IPMN is only in its infancy, and some unpublished preprints observed a high degree of heterogeneity in expression profiles and mucins at different pathological differentiation directions and degrees of differentiation in gastric, intestinal, and pancreaticobiliary types, suggesting that IPMN still has the potential to be subdivided [72,73].

ITPN

In more than 75% of ITPN cases, adenocarcinoma is also simultaneously found [131]. However, ITPN accounts for only 3% of pancreatic precancerous lesions and only 0.9% of all exocrine malignancies (Fig. 1) [132]. The most common location of ITPN is head of pancreas, which more often involved the main pancreatic duct/Wirsung duct (72.5%), or both main and branch pancreatic ducts (10%). Only 17.5% ITPN were observed along the branch pancreatic ducts [133]. Although associated invasive carcinoma is common, the prognosis of ITPN is much better than pancreatic cancer, with a 5-year survival rate of 71% in patients with ITPN-associated invasive carcinoma. Histologically, largely considered HG ITPN has a unique form of mucin expression, positive for MUC1 (> 90%) and MUC6 (70%), while lower levels of expression for MUC2 (8.6%), MCL amplifications (31.8%), FGFR2 fusions (18.2%), PI3KCA mutations (13.6%) are more common in ITPN (P < 0.001) [133]. The mechanism of progression of ITPN to pancreatic cancer is currently unclear, and Fukunaga *et al*. induced deletion of Arid1a and Pten in pancreatic ductal cells through activation of the YAP/TAZ pathway, leading to ITPN and ITPN-associated pancreatic malignancies [52]. ITPN most likely originates in the pancreatic ducts, unlike pancreatic cancer which originates in the classical pancreatic acinar cells. It also possesses rather different mutant forms [134]. Common pancreatic cancer driver genes (e.g., KRAS mutations (10.4%), TP53 mutations (4.7%), CDKN2A mutations (27.2%), and the gene SMAD4 mutations, GNAS mutations, RNF43 mutations) happened to have less mutations rates in ITPN [133]. In contrast, the relative lack of pancreatic cancer alterations in this case series highlights the molecular differences with conventional pancreatic cancer, but it should be acknowledged that KRAS alterations are still present in a non-negligible set of cases (still observed in 25% of patients with classical KRAS mutations) [131]. Approximately 30% of ITPNs have activating mutations in genes involved in the PI3K/Akt pathway (PIK3CA, PIK3CB, INPP4A, PTEN) and chromatin-remodeling factors (MLL1, MLL2, MLL3, BAP1, PBRM1, EED, ATRX, ARID2, ASXL1), suggesting that ITPN harbors distinct genetic alterations [135]. Some chromatin-remodeling factors were present only in the infiltrative component. As for chromosomal alterations, 1q increases (75%) and 1p, 6q or 18q deletions (about 50%) are the most common. As for structural variants, common fusions involve the recently identified RET and FGFR2. All ITPN and concurrent adenocarcinomas have most of the same genomic alterations [135].

MCN

MCNs of the pancreas are recognized for their mucin-producing capabilities and potential for malignant transformation. Their classification, mirroring PanINs and IPMNs, relies on the architectural and cytological atypia, segmenting them into low-grade (LG) or high-grade (HG). While LG MCNs predominantly feature gastric foveolar differentiation, HG MCNs exhibit pancreato-biliary traits. Notably, MCNs do not manifest the intestinal differentiation common in IPMNs. There is the absence of connections to the pancreatic duct system and the presence of a unique ovarian-type stroma beneath their neoplastic epithelium. This stroma is a diagnostic hallmark of MCNs. Interestingly, genetic investigations suggest an origin of MCNs from halted primordial germ cells, rather than standard pancreatic cells, elucidating their distinct cellular beginnings. This theory aligns with the clinical observations that MCNs predominantly affect females. From a genetic standpoint, early-stage MCNs frequently harbor mutations in the KRAS oncogene. Yet, as they progress, other genetic anomalies become evident. For instance, RNF43 mutations are prevalent in MCNs, reminiscent of IPMNs. However, GNAS mutations, a characteristic of IPMNs, remain absent in MCNs, highlighting their genetic disparity [136,137].

IOPN

IOPNs consist of complex dendrites lined with multiple

layers of cuboidal cells with granular eosinophilic granular stroma, round nuclei and prominent nucleoli. IOPNs were once classified as a subtype of IPMN, and they are now considered to be a distinct tumor. They are almost universally considered to have HG heterogeneous proliferation, and associated invasive carcinomas are common. But patients with IOPN are usually small and have a longer survival than pancreatic cancer. IOPNs lack somatic mutations in typical IPMN driver genes, but have been shown to have gene fusions involving PRKACA or PRKACB (Fig. 1) [138,139]. IOPNs are > 1 cm cystic nodular lesion with oncocytic features and ductal differentiation and are associated with pancreatic cancer in 60% of the cases, and IOPNs are frequently HG [127]. Detailed general descriptions of the tumors are available, most of which are described as multicompartmental or uni-compartmental cysts, some of which contain papillary projections or solid nodules. About 50% tumor is clearly associated with the main pancreatic duct. Microscopically, the tumor appeared as multifoveal or unifoveal cysts, with multifoveal and heterogeneous gross appearance. The tumor is arranged by multiple layers of tumor cells with abundant granular eosinophilic cytoplasm and large, fairly homogeneous nuclei containing a single distinct nucleolus. The 10-year overall survival of the whole cohort was 94%, and there was no difference between the invasive and noninvasive IOPN cohorts ($P = 0.38$) [140].

PanIN

Unlike other precancerous lesions of pancreatic cancer IPMN and MCN, both of which are macroscopic lesions, PanIN is defined as a microscopic, flat or shaped, noninvasive epithelial tumor that is < 5 mm in diameter on standard histological sections stained with hematoxylin and eosin and is characterized by varying amounts of mucin as well as cytological and structural degree of heterogeneity. It is considered to be the most common precancerous lesion of pancreatic cancer. However, the risk factors for PanIN are presumed to be similar to those for invasive pancreatic cancer. Thus, they would include old age, smoking, obesity, long-term diabetes, and chronic pancreatitis. Recent studies in mice have shown that pancreatic acinar cell carcinoma origin forms PanIN, whereas adenocarcinoma of ductal origin is not associated with the formation of PanIN. It is also possible to distinguish pancreatic cancer of ductal and follicular origin with high AGR2 [141].

Telomere shortening and activation site mutations in the KRAS oncogene appear to be among the earliest genetic alterations in PanIN lesions, as these genetic changes are present in most PanIN with LG heterogeneous hyperplasia. Inactivating mutations in the CDKN2A gene begin to appear in PanIN with low- to moderate-grade heterogeneous hyperplasia, and SMAD4 and TP53 mutations are common in HG PanIN, as they are largely only seen in PanIN with HG heterozygosis. The earliest alterations in pancreatic tumorigenesis are KRAS oncogenic hotspot mutations and telomere shortening, and a high prevalence occurs even in early lesions, with > 90% of LG PanINs having KRAS hotspot mutations and short telomeres. In contrast, the prevalence of alterations in key tumor suppressor genes (e.g., CDKN2A and TP53) in pancreatic tumorigenesis increases with increasing grade of heterozygosity, and the uniqueness of SMAD4 mutations in the landscape of pancreatic cancer is underscored by their rarity in PanINs, distinguishing them as a late-emerging genetic driver in the disease's progression. However, the absence of such mutations in a substantial percentage of pancreatic cases highlights the complexity and variability in the genetic underpinnings of this malignancy. PanINs may be intraductal extensions of invasive tumors and can be shown to be remote from the primary tumor in large numbers and discontinuously. HG PanINs are phylogenetically associated with infiltrative cancers and contain as many base substitutions as possible, but with fewer copy number alterations [142]. This can be explained by the colonization of PanINs through so-called ductal carcinogenesis, a phenomenon now reported clinically using histology [127,143]. The prevalence of chromosomal alterations and chromosome splitting increases with increasing PanIN grade, suggesting that aneuploidy may be a useful co-biomarker for driving mutations. In recent years, with the use of single-cell and spatial histology, the characteristics of PanIN are expected to be further revealed, with PanIN exhibiting increased expression of extracellular matrix-related genes (DCN, SPARC, and SPON1), a diversity of collagens, genes involved in ADM reprogramming (KLF4 and MMP7) and other markers of early-stage malignancy (CXCL12, TIMP3, ITGA1, and MUC5AC) [64]. The prevalence of LG precursor lesions is high, and in a recent Japanese autopsy series, LG PanINs were found in more than 75% of the population, and HG PanINs were found in approximately 5% of patients [144]. There was no difference in the prevalence of precursor lesions in patients with or without germline susceptibility [145]. As precancerous lesions can be completely removed surgically, PanINs represent promising potential target lesions for resection. However, there are challenges for lacking early detection methods.

Biomarkers

Pancreatic cancer is a progressive disease influenced by genetic mutations and epigenetic changes. From its initial mutation to clinical presentation, pancreatic cancer can

evolve over a span of 15–20 years [146]. However, the absence of accurate diagnostic techniques underscores the need to identify appropriate biomarkers for pancreatic cancer prevention and treatment. In recent years, advancements in artificial intelligence algorithms and various detection methods have significantly improved the discovery and utilization of biomarkers for pancreatic cancer. Recent advancements in artificial intelligence algorithms and diverse detection methodologies have greatly enhanced the identification and application of biomarkers in pancreatic cancer.

An ideal biomarker should offer consistent and effective diagnostic capabilities, be non-invasive, and monitor disease progression in real time [147]. For pancreatic cancer, CA19-9 stands as the sole United States Food and Drug Administration (FDA)-approved biomarker. Yet, it lacks both sensitivity and specificity, notably among patients with pancreatitis and those devoid of Lewis antigen expression [148]. Pathological criteria currently serve as the gold standard for diagnosing pancreatic cancer. Given the pancreas's distinct anatomical position and the challenges of replicating invasive procedures, choosing the right biomarkers has become a pivotal focus in contemporary pancreatic cancer research. Presently, sources such as blood, urine, pancreatic fluid, and gut microbiota offer potential biomarkers for pancreatic cancer. These biomarkers include various biological sample components like nucleic acids (for instance, circulating tumor DNA (ctDNA), cell-free circulating DNA (cfDNA), microRNAs (miRNA)) and their modifications (like DNA methylation), as well as proteins, peptides, cells (such as CTCs), and extracellular vesicles (like exosomes) [147,149]. Concurrently, recent research indicates that individual biomarkers' diagnostic capabilities remain insufficient. Pairing them with CA19-9 can boost their efficacy, which is a practice frequently adopted in current studies (Table 3).

Blood biomarkers

Blood receives raw materials, secretions, and waste products emitted by tumor cells. Thus, changes in blood composition are linked to the pathological processes of tumor cells. Blood is the most extensively researched source for biomarkers, encompassing types such as nucleic acids, proteins, lipids, and polysaccharides.

In recent years, there has been significant research on blood nucleic acids as potential biomarkers for pancreatic cancer. DNA fragments known as cfDNA are those present in blood's non-cellular components. They can result from the apoptosis and necrosis of tumor cells or healthy cells, as well as from the direct secretion of tumor cells or other microenvironmental cells, such as immune and inflammatory cells. The variable component of cfDNA produced by apoptotic or necrotic tumor cells is sometimes referred to as ctDNA, and it is recognized by certain cancer-related mutations [150,151]. Since ctDNA is directly derived from tumor cells, it has a high specificity and is significantly linked to tumor metastasis [150,151]. As a result, it is a valuable diagnostic tool that can predict a patient's prognosis. Although tumors have more cfDNA than healthy individuals, cfDNA detection is still tricky. To identify pancreatic cancer, the researcher applied the detection of cfDNA as a biomarker and paired it with CA19-9 and THBS2 (thrombospondin-2), which can achieve an impressive area under curve (AUC) of 0.94 [150]. Since KRAS mutation is prevalent in more than 90% of pancreatic cancer cases, ctDNA carrying the KRAS mutation has been extensively studied as a biomarker. Research has demonstrated that detecting KRAS mutation ctDNA is highly effective in predicting chemotherapy response and monitoring disease recurrence [152]. Recent investigations have indicated that mRNA [153,154], non-coding RNA (ncRNA) [146,155–157], tRNA-derived small RNA (tsRNA) [158,159], and other RNAs have high potential as biomarkers. Some of these RNA molecules are found in extracellular vesicles, which enhances their abundance and integrity as diagnostic markers. Moreover, the detection of nucleic acid alterations has emerged as a potent method in pancreatic cancer diagnosis. For instance, Majumder *et al.* developed a methylation DNA assay in combination with CA19-9, achieving an impressive AUC value of 0.97 [160].

With advancements in mass spectrometry analysis and enhanced detection techniques, our ability to detect various metabolites, including proteins, lipids, and oligosaccharides, has seen significant improvement recently. And research on the emergence of pancreatic cancer biomarkers based on this has also significantly increased.

The pancreatic cancer diagnosis model created by Kim *et al*. [161] and Mahajan *et al*. [162] utilized metabolic proteins and had an excellent AUC value. Asprosin was also employed by Nam *et al*. to efficiently and accurately diagnose pancreatic cancer [163]. The potential of lipids as a biological diagnostic tool for pancreatic cancer is very broad because the lipid changes may result from the tumor and tumor microenvironment cells and could also reflect the organism's immune response. Using the lipid model, Wolrab *et al*. can correctly identify pancreatic cancer [164]. CA19-9, an oligosaccharide secreted by tumor cells, has been approved by the FDA as a biomarker for pancreatic cancer. Yet, elevated levels of this substance are not universal among all patients. Considering this limitation, researchers have investigated an alternative oligosaccharide known as sialylated tumor-related antigen (sTRA) for its potential diagnostic replacement for CA19-9. The study findings suggest that

Table 3 Various pancreatic cancer biomarkers and diagnostic efficacy

Source	Type	Model	Specificty	Sensitivity	AUC	References
Blood	Oligosaccharide	sTRA	0.96	0.65	NA	164
	cfDNA	cfDNA	0.8	0.79	0.9	150
		cfDNA + CA19-9 + THBS2	0.93	0.92	0.94	150
	mRNA	GPC-1	1	1	1	154
	tsRNA	tRF-Pro-AGG-004	0.725	0.988	0.9	157
		tRF-Leu-CAG-002	0.64	0.772	0.78	157
		tRF-Pro-AGG-004 + tRF-Leu-CAG-002	0.964	0.85	0.94	157
	DNA	Methylated DNA markers	NA	NA	0.9	159
		Methylated DNA markers + CA19-9	0.92	0.92	0.97	159
	Protein	A multi-marker panel containing 14 proteins	0.959	0.904	0.977	160
		A multi-marker panel containing 14 proteins + CA19-9	0.983	0.927	0.989	160
		i-Metabolic (12 analytes + CA19-9)	0.877	0.908	0.972	161
		m-Metabolic (4 analytes + CA19-9) with machine learning–aided algorithm	0.896	0.773	0.904	161
		Asprosin	0.924	0.957	0.987	162
	Lipid	Lipidomic profiling	>0.94	>0.94	0.983	163
		Lipidomic profiling + CA19-9	NA	NA	0.989	163
Urine	Protein	3 protein biomarkers (LYVE1 + REG1B + TFF1)	0.919	0.725	0.936	164
		3 protein biomarkers (LYVE1 + REG1B + TFF1) + CA19-9	NA	NA	0.992	164
Pancreatic juice	miRNA	miR-16 + miR-21 + miR-25 + miR-155 + miR-210 + CA19-9	84.2	81.5	0.91	166
Faecal	Microbiota	Faecal metagenomic classifiers based on a set of 27 microbial species	NA	NA	0.84	167
		Faecal metagenomic classifiers based on a set of 27 microbial species + CA19-9	NA	NA	0.94	167
	Microbiota	30 gut microbiomes	NA	NA	0.78–0.82	155
		30 gut microbiomes + CA19-9	NA	NA	0.81–0.97	155

Abbreviations: cfDNA, cell-free circulating DNA; AUC, area under curve; tsRNAs, tRNA-derived small RNAs; miRNAs, microRNAs; CA19-9, carbohydrate antigen 19-9; NA, not available; sTRA, sialylated tumor-related antigen.

sTRA exhibits comparable diagnostic potential to CA19-9 in clinical patients [165].

Urine biomarkers

Although urine biomarkers have received less attention as a diagnostic method for pancreatic cancer, they hold significant promise. The kidneys' ultrafiltration function makes urine an advantageous source for biomarker analysis, offering benefits such as easy sample collection, reproducible procedures, higher concentration, and reduced variability. Notably, Debernardi *et al*. has developed a diagnostic model for pancreatic cancer utilizing three proteins that exhibit stable expression in urine and bind to CA19-9, yielding an impressive AUC of 0.992 [166].

Pancreatic fluid biomarkers

Pancreatic fluid holds great potential as a source of biomarkers for pancreatic cancer due to its proximity to the ductal cells that produce PDAC. As the pancreas directly secretes pancreatic fluid, it will likely contact tumor cells directly, resulting in a higher specificity than other sample sources. With advancements in duodenoscopy and endoscopic ultrasound (EUS), obtaining pancreatic fluid has become safer. Nesteruk *et al*. discovered that pancreatic fluid exhibited higher sensitivity than serum for pancreatic cancer diagnosis. In addition, they found that miRNA present in extracellular vesicles of pancreatic fluid combined with CA19-9 was effective in diagnosing pancreatic cancer with a diagnostic AUC value of 0.91 [167].

Gut microbiota biomarkers

The connection between gut microbiota and the pancreas is evident, with notable alterations in the gut microbiota of pancreatic cancer patients compared to healthy individuals. These changes in gut microbiota are

intricately involved in the progression of pancreatic cancer, highlighting its potential as a biomarker for this disease. Kartal *et al*. developed a predictive model utilizing 27 species of gut microbiota combined with CA19-9, which achieved an impressive AUC of up to 0.94 for pancreatic cancer detection [168]. Furthermore, Nagata *et al*. employed machine learning algorithms to establish a diagnostic model for pancreatic cancer based on gut microbiota, demonstrating significant diagnostic efficacy. This model was further validated in Germany, Spain, and Japan [155].

Chemoresistance

Given the challenges in diagnosing pancreatic cancer early on, a majority of patients are identified with advanced tumors or metastases, leaving only about 20% with surgical options [3]. Most patients require chemotherapy, among which GEM-based chemotherapy is the first-line chemotherapy regimen for pancreatic cancer [169,170]. However, chemoresistance limits the clinical outcomes of chemotherapy (Fig. 2).

TME

The TME plays a pivotal role in the formation and progression of tumors. Specifically, the TME of pancreatic cancer is marked by a dense intercellular matrix and a notable accumulation of extracellular matrix [171]. Pancreatic cancer cells are encapsulated within a thick fibrotic matrix [172]. Excessive fibrosis around the tumor cells and a large majority of interstitial components increases the interstitial fluid pressure (IFP), reaching more than 10 times higher than in healthy pancreas. The formation of a high-pressure barrier around the tumor cells prevents chemotherapy treatments from reaching the tumor cells because it reduces blood flow to the tumor cells, results in blood vessel collapse, and inhibits the

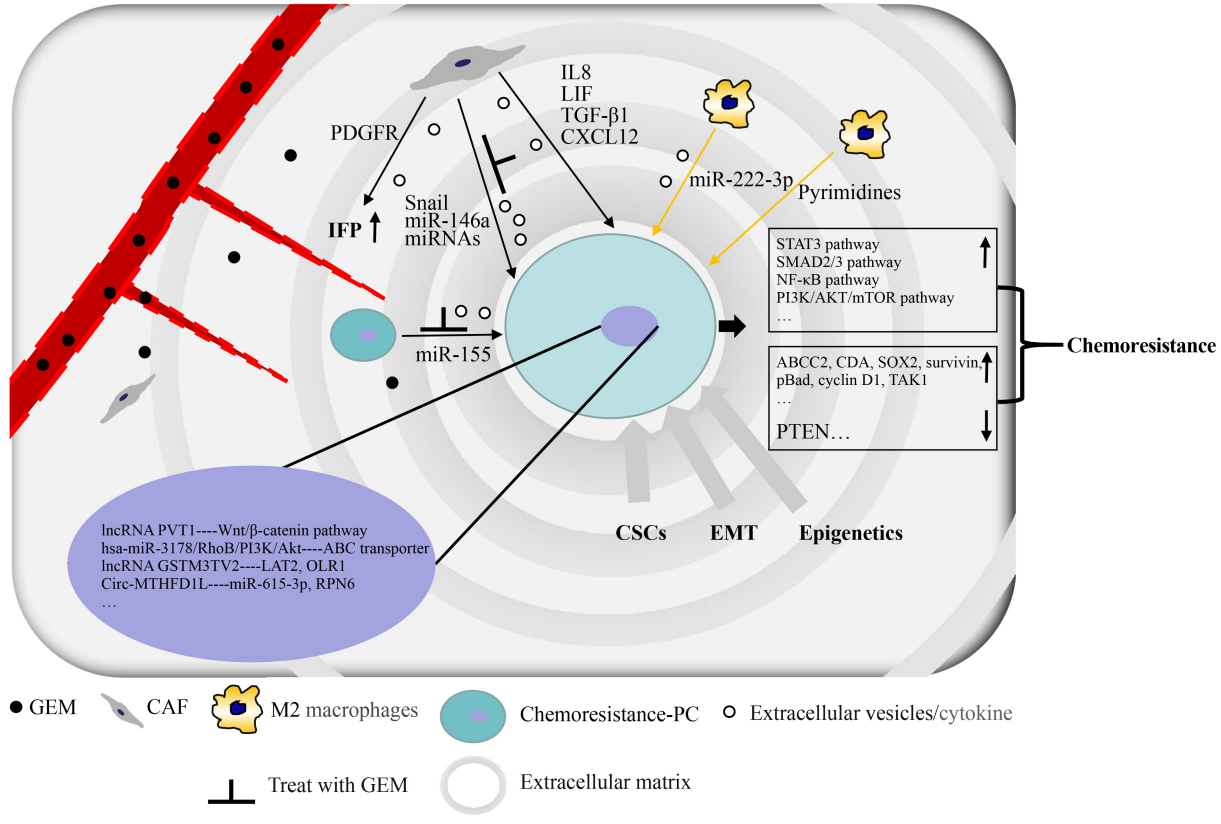

Fig. 2 Formation of chemoresistance in pancreatic cancer. The pancreatic cancer tumor microenvironment is involved in the formation of chemoresistance in pancreatic cancer: (1) The massive accumulation of extracellular matrix in pancreatic cancer TME causes an increase in the IFP, forming a high-pressure barrier outside the cells. This not only inhibits drug penetration but also leads to tumor cells being in ischemic, hypoxic and acidic conditions, prompting metabolic reprogramming of tumor cells, which in turn facilitates the development of chemoresistance; (2) The emergence of crosstalk between multiple cells in pancreatic cancer TME promotes the development of chemoresistance in pancreatic cancer. Alterations in the mechanisms of pancreatic cancer cells themselves are involved in the development of chemoresistance in pancreatic cancer: (1) lncRNAs, circRNAs, and miRNAs are involved in chemoresistance related to pancreatic cancer; (2) CSCs, EMT, and epigenetic changes are involved in chemoresistance in pancreatic cancer. Abbrevations: IFP, interstitial fluid pressure; CSCs, cancer stem cells; EMT, epithelial-mesenchymal transition; TME, tumor microenvironment; lncRNAs, long non-coding RNAs; circRNAs, circular RNAs; miRNAs, microRNAs; GEM, gemcitabine.

penetration of chemotherapy drugs [173]. Additionally, this situation exposes tumor cells to ischemic, hypoxic, and acidic conditions, triggering their metabolic reprogramming and elevating their resistance to chemotherapy [171].

Beyond causing physical alterations due to elevated interstitial pressure and its ensuing effects, interstitial elements in the tumor microenvironment also promote the chemotherapy resistance of tumor cells, including fibroblasts, pancreatic astrocytes, extracellular matrix, and immune cells [174].

By secreting exosomes and cytokines, interstitial cells help tumor cells develop resistance to chemotherapy [175]. By secreting stromal platelet-derived growth factor receptor (PDGFR), stromal fibroblasts cause interstitial contracture and exacerbate interstitial hypertension [176]. Hu *et al*. discovered that CAFs elevate LIF secretion, thereby activating the STAT3 signaling pathway in pancreatic cancer cells [177]. This increase heightens the expression of proteins tied to GEM resistance like ABCC2, CDA, and SOX2, leading to the rise of GEM-resistant pancreatic cancer cells [177]. Additionally, CAFs can release TGF-1, IL-8, CXCL12, and other substances that activate the SMAD2/3, NF-κB, and other chemotherapy resistance-related pathways [178–180]. Besides, M2 macrophages can generate extracellular vesicles, which can be used to deliver miR-222-3p to tumor cells and activate the PI3K/AKT/mTOR pathway, which increases chemoresistance [181]. Tumor-associated macrophages (TAM) can induce GEM resistance by secreting pyrimidine and competitively inhibiting GEM [182]. Laminin (LN), a protein of the extracellular matrix, induces FAK phosphorylation in pancreatic cancer cells, resulting in Akt phosphorylation and increased expression of apoptosis-related proteins (increasing survivin and pBad (pS136) levels), thereby inducing GEM resistance in pancreatic cancer [183].

Moreover, upon exposure to chemotherapy, these resistant cells further drive the emergence of chemotherapy-resistant counterparts through the secretion of exosomes and cytokines. Richards *et al*. discovered that GEM treatment could increase the expression of Snail and Snail target microRNA-146a in CAF and transfer these proteins to tumor cells through exosomes to promote GEM-resistance in tumor cells [184]. Richards *et al*.'s study found that CAFs exposed to GEM release exosomes containing miRNAs (miR-21, miR-181a, miR-221, miR-222, and miR-92a) that act on PTEN in tumor cells, which can lead to chemoresistance [185]. This interplay of chemoresistance is also evident between tumor cells. Patel *et al*. observed that upon GEM treatment, pancreatic cancer cells amplify exosome secretion. This results in the transfer of miR-155 to drug-sensitive pancreatic cancer cells, subsequently suppressing DCK expression and fostering chemoresistance [186].

Formation of chemoresistance mechanism in pancreatic cancer

Pancreatic cancer may experience mechanism modifications to formate chemoresistance in addition to TEM alterations in pancreatic cancer [187]. Recent studies have revealed that chemoresistance can arise from cancer stem cell (CSC), EMT, and epigenetics. According to Cioffi *et al*.'s research, the percentage of CSC is inversely correlated with the tumor's chemoresistance, and some treatment strategies specifically targeting CSC are more effective in preventing the development of chemoresistance [188,189]. Xiaofeng Zheng suppressed EMT by creating transgenic mice with EMT-induced transcription factor (Snail or Twist) knockouts and discovered that balance nucleoside transporter (ENT1) and concentrated nucleoside transporter (Cnt3) were dramatically elevated after EMT inhibition, and it can significantly increase the sensitivity of GEM [190]. m6A methylation stands out as the predominant epigenetic modification. GEM chemoresistance can be prevented by ALKBH5, an m6A demethylase, via transactivating WIF-1 and subsequently inhibiting Wnt signaling [191]. By facilitating the upregulation of DDIT4-AS1 by activating the mTOR pathway, it can also preserve the stemness of pancreatic cancer and decrease chemosensitivity.

Analyzing the differentially expressed genes (DEGs) across pancreatic cancer cell lines with distinct drug resistance profiles revealed that these DEGs predominantly modulate GEM metabolism transporters, cell cycle regulation, and signaling pathways associated with proliferation or apoptosis [192]. Furthermore, a spectrum of ncRNAs, encompassing long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and miRNAs, have emerged as regulators of chemoresistance in pancreatic cancer. As highlighted by Gu *et al*., the elevated expression of ABC transporter proteins, steered by the hsa-miR-3178/RhoB/PI3K/Akt axis, culminates in GEM resistance in pancreatic cancer cells [193]. According to Tsai-Fan Chou, lncRNA PVT1 enhances autophagy activity and Wnt/β-catenin pathway signaling to promote GEM resistance in pancreatic cancer [194]. lncRNA GSTM3TV2 competitively regulates let-7, upregulating LAT2 and OLR1, thereby increasing GEM resistance in pancreatic cancer [195]. The Circ-MTHFD1L/miR-615-3p/RPN6 signaling axis maintains GEM resistance in pancreatic cancer [196]. Upregulation of vASH2 leads to JUN induction, which activates RRM2 transcription through direct binding to the RRM2 promoter, resulting in GEM resistance [197]. Furthermore, researchers have found that GEM increases the expression of cyclin D1 and TAK1 in pancreatic cancer, promoting cell proliferation and inhibiting drug-induced apoptosis, consequently leading to

chemoresistance [198,199]. Activation of the EGFR self-activation (phosphorylation), mTOR signaling pathway, NF-κB signaling pathway, and STAT3 signaling pathway in pancreatic cancer also plays a crucial role in promoting the development of chemoresistance [200,201].

Immunotherapy resistance

Immunotherapy is a new direction of tumor therapy. Currently, immunotherapy encompasses adoptive cell therapy (such as chimeric antigen receptor T cells (CAR-T)) [202,203], oncolytic vaccines, and immune checkpoint blocking (ICB) [204]. Immunotherapy has got positive survival outcomes in patients with advanced solid malignancies, such as melanoma and lung cancer. However, directly transplanting this therapeutic approach is not effective for pancreatic cancer, a challenge attributed to the immunotherapy resistance of this cancer type (Fig. 3).

Pancreatic cancer is characterized by its low immunogenicity and suboptimal antigen presentation [205]. The density of its tumor-associated antigens (TAAs) is markedly diminished compared to malignancies like melanoma, and its tumor mutation burden (TMB) also lags, posing significant challenges to igniting anti-tumor immune responses [206,207]. Additionally, the lack of mature DC cells in pancreatic cancer can lead to poor antigen presentation, which inhibits T cell activation [208].

Concurrently, there is a notable redistribution of immune cells within the TME of pancreatic cancer [209]. T lymphocytes play an essential role in anti-tumor immunity, and T cells in TME include $CD8^+$ cytotoxic T cells (CTL), also known as effector T cells [210]. $CD4^+$ T cells include helper T cells (Th) Th1, Th2, Th17, Tregs. CTL and Th1 $CD4^+$ T cells are conducive to anti-tumor immunity. However, in TMEs of pancreatic cancer, the distribution of CTL is infrequent, and this scarcity

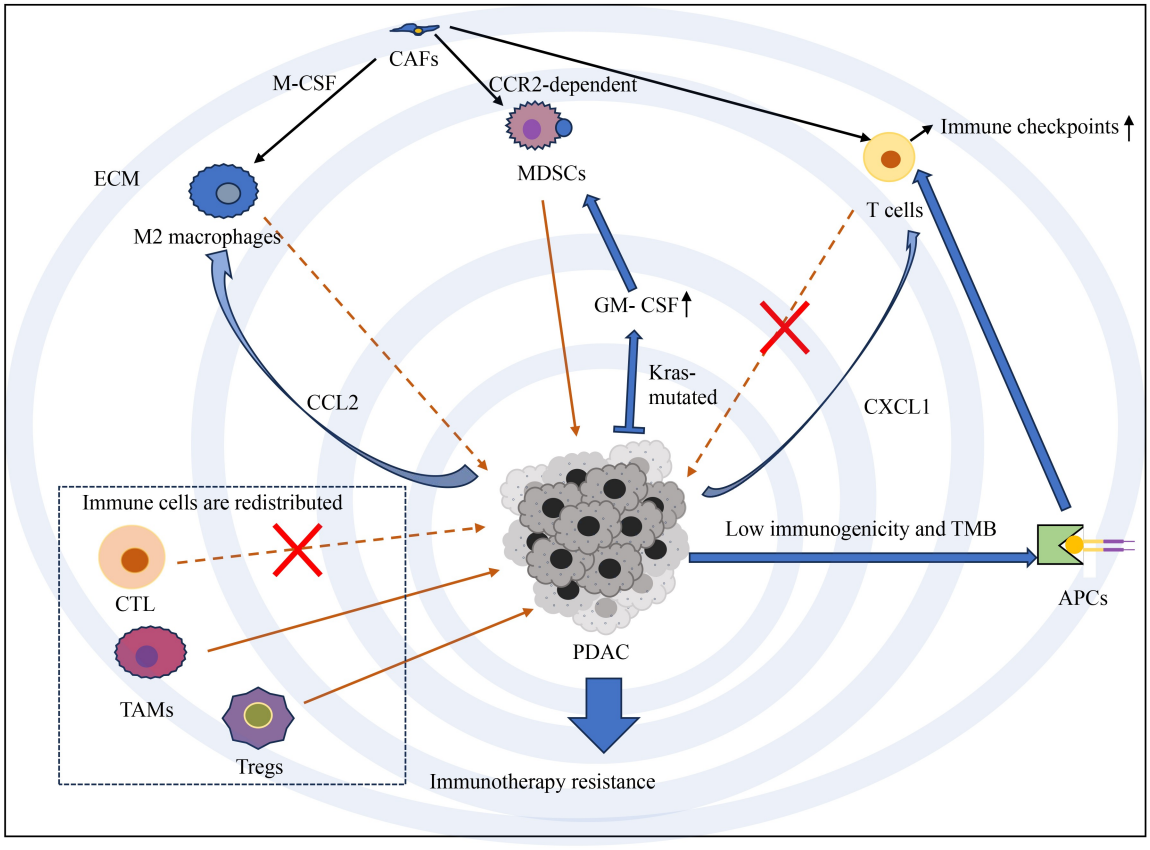

Fig. 3 The formation of immunotherapy resistance in pancreatic cancer. (1) Pancreatic cancer cells themselves exist with low immunogenicity and TMBs. (2) There exists a high-pressure barrier around tumor cells due to the accumulation of large amounts of extracellular matrix. (3) The presence of crosstalk between multiple cells (tumor cells, CAF, immune cells) in the TME promotes the formation of a tumor immunosuppressive microenvironment. Abbrevations: CAFs, cancer-associated fibroblasts; TMB, tumor mutational burden; GM-CSF, granulocyte-macrophage colony-stimulating factor; APCs, antigen-presenting cells; MDSCs, myeloid-derived suppressor cells; CTL, cytotoxic T lymphocyte; TAMs, tumor-associated macrophages; Tregs, regulatory T cells; PDAC, pancreatic ductal adenocarcinoma; ECM, extracellular matrix; M-CSF, macrophage colony-stimulating factor; CCL2, chemokine (C-C motif) ligand 2; CCR2, C-C chemokine receptor type 2; CXCL1, C-X-C motif chemokine ligand 1.

becomes increasingly pronounced as one approaches the tumor [206]. Furthermore, the TME experiences an influx of numerous immunosuppressive cells, encompassing entities like TAMs, myeloid-derived suppressor cells (MDSCs), and Tregs, etc. [211], which can inhibit the body's anti-tumor immune response by inhibiting the killing function of natural killer (NK) cells, DCs, effector T cells, and other cells. At the same time, these immune cells will appear to crosstalk [212]. The net result further inhibits the distribution and infiltration of immuno-suppressive effector cells and increases the proportion of immunosuppressive cells [207].

TME stromal cells and pancreatic cancer cells can release cytokines that alter immune system components. To be specific, KRAS-mutated tumor cells have the capacity to upregulate granulocyte-macrophage colony-stimulating factor (GM-CSF) and MDSC chemotaxis [213]. CCL2 secreted by pancreatic cancer promotes macrophage infiltration [214]. CXCL1 secreted by tumor cells can cause an increase in bone marrow cells and a decrease in cytotoxic $CD8^+$ T cell infiltration [215]. In addition, CAFs can also mediate the upregulation of immune checkpoints in T cells to inhibit adaptive immunity and can secrete macrophage colony-stimulating factor (M-CSF) to promote M2 polarization of macrophages [216] and promote the recruitment of MDSC in a CCR2-dependent manner [217].

Treatment of pancreatic cancer

The outlook for pancreatic cancer remains deeply concerning. Although the basic research and treatment methods of pancreatic cancer have made breakthroughs in recent years, the 5-year survival rate is only 10%. At present, surgical resection combined with systemic chemotherapy is the only way for pancreatic cancer patients to survive for a long time. And the main therapeutic methods for pancreatic cancer patients are surgical therapy, chemotherapy, targeted therapy, immunotherapy, etc. [218]. At the same time, the therapeutic concept is developing in the direction of multi-discipline and individual.

Owing to the pancreas's intricate anatomical position, pancreatic surgery presents formidable challenges. Undertaking a surgical resection for pancreatic cancer demands unparalleled surgical acumen and extensive perioperative care expertise [219]. Perioperative mortality has been lowered to 3%–4% owing to ongoing advancements in pancreatic surgery methods and techniques [220]. As surgical protocols for pancreatic cancer enhance safety, a broader cohort of patients now becomes eligible for surgical intervention compared to the past. Recent research has shown that surgical treatment, particularly the excision of R0, can improve survival outcomes in patients with pancreatic cancer who have locally advanced stage [221], borderline resectable [222], and distant oligo metastases (such as liver/distant lymph node metastasis) [223,224]. Due to pancreatic surgery's high difficulty and complications, many medical centers will prudently select surgical patients, especially some older adults. However, the latest research shows that aging does not affect the long-term survival of pancreatic cancer patients undergoing surgical treatment [225]. At the same time, the emergence of neoadjuvant therapy can transform some patients from unresectable to having the chance of surgery, realizing the downtime, and improving the proportion of R0 excision [221,222].

The impact of surgical interventions and associated complications on the duration and efficacy of chemotherapy has remained a subject of ongoing debate. The recent ESPAC-3 study indicated that, as long as patients can finish the entire chemotherapy plan, their prognosis will not be impacted even if postoperative chemotherapy is started 12 weeks after surgery instead of the traditional 6 weeks after surgery [226]. Advancements in minimally invasive techniques, encompassing laparoscopic and robotic procedures, promise reduced patient discomfort, shorter hospital stays, and expedited recovery, all while maintaining surgical safety [227–229].

Over the past several decades, GEM has emerged as the cornerstone chemotherapy regimen for pancreatic cancer patients. With the deepening of research in recent years, the current first-line chemotherapy regimen is FOLFIRINOX and GEM plus albumin combined with paclitaxel [230]. However, there is no doubt that the side effects of chemotherapy and chemoresistance limit the prognosis of patients with pancreatic cancer. At present, some chemotherapy-based combination therapy strategies have been proposed with encouraging results, but it will take a significant amount of time to clinically verify these schemes (Table 4).

The intricate pathological landscape of pancreatic cancer is predominantly governed by gene mutations, which hold pivotal roles in the disease's onset and progression. Currently, KRAS, TP53, CDKN2A, and SMAD4 have been identified as the four primary driving genes involved in the whole process of disease development [231]. Additionally, Sian Jones reported 12 core signaling pathways enriched in mutated genes in pancreatic cancer and proposed that regulating these signaling pathways is also a direction for treatment [232]. All these also provide a theoretical basis for the targeted therapy of pancreatic cancer (Table 4). However, the progress of targeted therapy is limited in pancreatic cancer, and the vast majority of targeted therapy trials have failed, which is closely related to the complexity and compensation of mechanism regulation [233].

Immunotherapy is a new direction of current treatment,

Table 4 Clinical trials of treatments for PC

Theme	Target	Included population	Approach	Time	Effectiveness	DOI	Conclusion
Gem-based clinical trials	NA	Advanced PC patients	GEM+5-FU	2002	OS: 6.7 Ms; PFS: 2.2 Ms	10.1200/JCO.2002.11.149	(1) GEM-based chemotherapy can bring survival outcomes to patients with advanced PC and patients with operable PC; (2) FOLFIRINOX can bring more survival benefits than GEM, but FOLFIRINOX is more demanding for patients; (3) GEM can improve the efficacy of 5-FU-based adjuvant chemoradiotherapy, and radiotherapy can improve the therapeutic effect of GEM
			GEM		OS: 5.4 Ms; PFS: 2.2 Ms		
		Advanced PC patients	pemetrexed+GEM	2005	OS: 6.2 Ms; PFS: 3.9 Ms	10.1093/annonc/mdi309	
			GEM		OS: 6.3 Ms; PFS: 3.3 Ms		
		Patients with complete surgical resection of PC	Postoperative: GEM	2007	MDFS: 13.4 Ms; DFS at 3 years:23.5%; DFS at 5 years:16.5%; during a median follow-up of 53 Ms, 74% had relapsed	10.1001/jama.297.3.267	
			Postoperative: untreated		MDFS: 6.9 Ms; DFS at 3 years: 7.5%; DFS at 5 years: 5.5%; during a median follow-up of 53 Ms, 92% had relapsed		
		Patients with locally advanced or metastatic PC	Postoperative: GEM+5-FU+radiotherapy	2008	OS: 20.5 Ms; 3-year survival: 31%	10.1001/jama.299.9.1019	
			Postoperative: 5-FU+radiotherapy		OS: 16.9 Ms; 3-year survival: 22%		
		Patients with locally advanced or metastatic PC	GEM	2010	OS: 8.3 Ms; PFS 3.9 Ms; ORR: 10.1%; CB: 23.0%	10.1200/JCO.2009.25.4433	
			GEM+cisplatin		OS: 7.2 Ms; PFS 3.8 Ms; ORR: 12.9%; CB:15.1%		
		Patients with inoperable PC	Enzyme-therapy	2010	OS: 14 Ms; 1-year survival: 56%	10.1200/JCO.2009.22.8429	
			GEM-based chemotherapy		OS: 4.3 Ms; 1-year survival: 16%		
		Patients with metastatic PC	FOLFIRINOX	2011	OS: 11.1 Ms; PFS: 6.4 Ms	10.1056/NEJMoa1011923	
			GEM		OS: 6.8 Ms; PFS: 3.3 Ms		
		Patients with unresectable PC	Radiotherapy+GEM	2011	OS: 9.2 Ms	10.1200/JCO.2011.34.8904	
			GEM		OS: 11.1 Ms		
		Patients with complete surgical resection of PC	GEM	2013	MDFS: 13.4 Ms; 5-year survival: 20.7%; 10-year survival: 12.2%	10.1001/jama.2013.279201	
			Untreated		MDFS: 6.7 Ms; 5-year survival: 10.4%; 10-year survival: 7%		
		Patients with surgical resection of PC	Modified-FOLFIRINOX	2018	MDFS :21.6 Ms; OS: 54.4 Ms	10.1056/NEJMoa1809775	
			GEM		MDFS: 12.8 Ms; OS: 35 Ms		
Targeted therapy related clinical trials	LOX and TBXAS	Advanced PC patients	CV6504	2000	1-year survival: 25%.	10.1023/a:1008303715515	Most of the current clinical trials of targeted therapy are unsuccessful. Even a few of them have effects, but when they enter phase III clinical trials, they cannot achieve ideal results
	Matrix metalloproteinases	Unresectable PC patients	Marimastat	2001	OS: 125 Ds; 1-year survival: 20%	10.1200/JCO.2001.19.15.3447	
	Thymidylate synthase	Advanced PC patients	GEM+Tomudex	2003	OS: 185 Ds	10.1093/annonc/mdg150	
	FTase	Advanced PC patients	R115777	2003	PFS: 4.9 Ws; OS: 19.7 Ws	10.1200/JCO.2003.08.040	
	mTOR pathway	Patients with gemcitabine-refractory, metastatic PC	RAD001 (everolimus)	2009	PFS: 1.8 Ms; OS: 4.5 Ms	10.1200/JCO.2008.18.9514	

(*Continued*)

Theme	Target	Included population	Approach	Time	Effectiveness	DOI	Conclusion
	EGFR	Patients with R0- or R1-PC	GEM+cetuximab	2013	OS: 22.4 Ms; DFS: 10.0 Ms	10.1093/annonc/mdt270	
	IGF1R pathway	Patients with previously untreated metastatic PC	GEM	2015	OS: 7.2 Ms	10.1093/annonc/mdv027	
			Ganitumab+GEM		OS: 7.0 Ms		
	The MEK and PI3K/AKT pathways	Patients with gemcitabine-refractory, metastatic PC	Selumetinib+MK-2206	2017	OS: 3.9 Ms; PFS: 1.9 Ms	10.1001/jamaoncol.2016.5383	
			mFOLFOX (oxaliplatin plus fluorouracil)		OS: 6.7 Ms; PFS: 2 Ms		
	Src kinase	Patients with locally advanced, non-metastatic PC	GEM+dasatinib	2017	OS: 375 Ds; PFS: 167 Ds	10.1093/annonc/mdw607	
			GEM		OS: 393 Ds; PFS: 166 Ds		
	EGFR	Untreated, unresectable, advanced/metastatic PC patients	GEM+nimotuzumab	2017	OS: 8.6 Ms; PFS: 5.1 Ms	10.1093/annonc/mdx343	
			GEM		OS: 6.0 Ms; PFS: 3.4 Ms		
	The EGFR tyrosine kinase	Patients with resectable PDAC post-R0 resection	GEM+erlotinib	2017	OS: 24.5 Ms; DFS: 11.4 Ms	10.1200/JCO.2017.72.6463	
			GEM		OS: 26.5 Ms; DFS: 11.4 Ms		
	Wee1 kinase	Newly diagnosed, locally advanced PC patients	AZD1775 (adavosertib)+GEM+radiation	2019	OS: 21.7 Ms; DFS: 9.4 Ms	10.1200/JCO.19.00730	
	FTase	Advanced PC patients	Tipifarnib+GEM	2004	OS: 193 Ds; PFS: 112 Ds	10.1200/JCO.2004.10.112	
			GEM		OS: 182 Ds; PFS: 108 Ds		
	Ras-dependent signaling and angiogenic pathways	Advanced PC patients	GEM+sorafenib	2012	OS: 8 Ms; PFS: 3.8 Ms; 6-month PFS: 33%	10.1093/annonc/ mds135	
			GEM		OS: 9.2 Ms; PFS: 5.7 Ms; 6-month PFS: 48%		
	JAK/STAT pathway	Patients with gemcitabine-refractory, metastatic PC	Ruxolitinib+capecitabine	2015	OS: 4.5 Ms	10.1200/JCO.2015.61.4578	
			Capecitabine		OS: 4.3 Ms		
	SHH	PC patients not suitable for curative treatment with no prior metastatic therapy	Vismodegib+GEM	2015	OS: 6.9 Ms; PFS: 4.0 Ms	10.1200/JCO.2015.62.8719	
			GEM		OS: 6.1 Ms; PFS: 2.5 Ms		
	VEGF-A	Advanced PC patients	GEM+bevacizumab	2010	OS: 5.8 Ms	10.1200/JCO.2010.28.1386	
			GEM		OS: 5.9 Ms		

Abbreviations: PC,pancreatic cancer; OS, overall survival; PFS, progression free survival; MDFS, median disease-free survival; ORR; objective response rate; CB, clinical benefit; 5-FU, 5-fluorouracil; GEM, gemcitabine; FTase, farnesyltransferase; EGFR, epithelial growth factor receptor; SHH, sonic hedgehog; VEGF-A, vascular endothelial growth factor A; Ms, months; Ds, days; Ws, weeks; JAK/STAT pathway, Janus kinase/signal transducer and activator of transcription pathway; LOX, lipoxygenase; TBXAS, thromboxane A2 synthetase; PI3K/AKT, phosphoinositide 3-kinase/protein kinase B; FOLFIRINOX, fluorouracil+leucovorin+irinotecan+oxaliplatin; NA, not applicable; PDAC, pancreatic ductal adenocarcinoma.

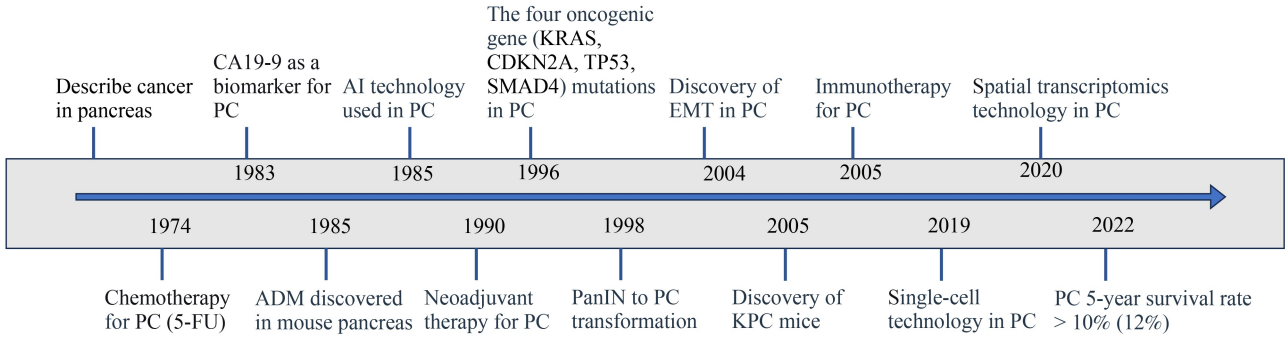

Fig. 4 Timeline of pancreatic cancer progression. Abbrevations: PC, ancreatic cancer; AI, artificial intelligence; ADM, acinar-to-ductal metaplasia; PanIN, pancreatic intraductal neoplasias; EMT, epithelial-mesenchymal transition.

which has improved the survival of patients with advanced solid tumors, including melanoma and lung cancer [234–236]. However, the outcomes of pancreatic cancer have been disheartening [218]. Current immunotherapy in pancreatic cancer includes immune checkpoint suppression therapy, pancreatic cancer immune vaccine, and CAR-T infusion, but these outcomes are poor [237,238].

Treatment with immune checkpoint inhibitors, including PD-L1 and CTLA-4, has failed but has shown promising results in a subset of patients with microsatellite instability, indicating a direction for immunotherapy in pancreatic cancer. Furthermore, based on the fact that previous immunotherapy has little clinical activity, there are also several multimodal immunotherapies that are being tried, such as chemotherapy/radiotherapy combined immunotherapy, and some good clinical results have been obtained [206,239].

Conclusions

As of now, the advancements in chemotherapy and targeted treatments have modestly elevated the 5-year survival rate for pancreatic cancer patients, rising from a mere 2% a decade ago to 12% in 2022. And our deepened comprehension of pancreatic cancer's subtype biology paves the way for a more nuanced and targeted therapeutic approach (Fig. 4). New clinical trial designs, including drug introductions, new adjunctive tests for investigational drugs, and platform studies that allow for rapid test combinations, are facilitating progress. Pancreatic cancer encompasses a diverse array of malignant epithelial neoplasms, characterized by intricate histological configurations and a varied genetic and molecular landscape. This malignancy arises from multiple precursor lesions, notably PanIN, IPMN, IOPN, ITPN, and MCN. The latest molecular categorization of pancreatic cancer, informed by comprehensive genomic, transcriptomic, proteomic, and epigenetic analyses, offers critical insights into its molecular diversity and the inherently aggressive nature of the disease. Investigations at the single-cell level have shown significant promise, surmounting earlier challenges arising from the amalgamation of stromal and tumor cells. However, there exist several pressing questions that still await comprehensive answers. While single-cell analyses have yet to establish a molecular classification that deeply informs clinical protocols, they are also hampered by the need for fresh tissue samples, affecting the quality and collaborative scope of research due to subpar cell dissociation and RNA integrity. This issue contributes to an insufficient capture of stromal cells in pancreatic cancer samples, skewing cell type representation. Though neoadjuvant therapies show promise, a significant knowledge gap persists as current genomic studies typically concentrate on pre-treatment conditions. Investigating the genomic consequences of treatment, particularly through comparative pre- and post-therapy mRNA studies, is crucial. The practical impact of molecular findings hinges on their clinical applicability, necessitating a focus on cell-specific biology, intercellular interactions, and tumor behavior over time to craft targeted treatment plans. Despite the nascency of precision oncology in pancreatic cancer, the anticipation is building for trials utilizing specific molecular categorizations and markers, signaling a shift away from uniform treatment methods.

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Compliance with ethics guidelines

Conflicts of interest Zhichen Jiang, Xiaohao Zheng, Min Li, and Mingyang Liu declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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