Review

Molecular Biomarkers of Pancreatic Intraepithelial Neoplasia and Their Implications in Early Diagnosis and Therapeutic Intervention of Pancreatic Cancer

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Abstract

Lack of early detection and effective interventions is a major reason for the poor prognosis and dismal survival rates for pancreatic cancer. Pancreatic intraepithelial neoplasia (PanIN) is the most common precursor of invasive pancreatic ductal adenocarcinoma (PDAC). Each stage in the progression from PanIN to PDAC is well characterized by multiple significant genetic alterations affecting signaling pathways. Understanding the biological behavior and molecular alterations in the progression from PanIN to PDAC is crucial to the identification of noninvasive biomarkers for early detection and diagnosis and the development of preventive and therapeutic strategies for control of pancreatic cancer progression. This review focuses on molecular biomarkers of PanIN and their important roles in early detection and treatment of pancreatic cancer.

Key words: PanINs; Biomarker; Early Diagnosis; Therapeutic Intervention

Introduction

The overall 5-year survival rate in patients with pancreatic cancer is about 5%, and this disease is the fourth leading cause of cancer deaths in the United States [1]. Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer. Its high mortality rate is partly a result of difficulty in establishing an effective strategy for early detection and diagnosis and a lack of effective treatments of advanced-stage disease. The asymptomatic nature of early-stage pancreatic cancer and remote anatomic location of the pancreas increase the difficulty in early detection.

Main diagnostic procedures for PanINs include histopathological diagnosis, genetic diagnosis (detection of gene mutation) [2], imaging diagnosis, such as B-ultrasound, CT and MRI, detection of serum tumor markers, biopsy of circulation tumor DNA (ctDNA)

and circulation tumor cell (CTC) in a noninvasive manner [3,4]. At present, histopathological diagnosis remains the most accurate and reliable gold standard of the diagnosis of PanINs. The accuracy of different diagnostic methods greatly varies. The sensitivity, specificity and accuracy of CA19-9 detection in the diagnosis of pancreatic cancer were 83.1%, 73% and 75%, respectively. Approximately 99% of *KRAS* gene mutation is intimately correlated with the incidence and progression of early lesions induced by pancreatic cancer. A substantial quantity of early pancreatic cancer can be diagnosed in a noninvasive manner by biopsy detection of ctDNA. Melo and colleagues have recently demonstrated that glypican-1 (GPC1) gene encoding protein, distributed in the cancer exosomes, can serve as a potential marker for noninvasive diagnosis and screening tool [4].

Authors reported that progression from preinvasive precursor lesions to invasive pancreatic cancer occurs over many years or decades, and the time required for parental pancreatic cancer to gain the capacity to invade and metastasize is typically more than 5 years [5]. Pancreatic intraepithelial neoplasias (PanINs) are well established as the most common precursors of PDAC [6]. PanINs are microscopic lesions (<5 mm in diameter) that are too small to be identified using current imaging techniques. Multiple PanINs are usually found in individuals with inherited susceptibility to pancreatic cancer [7, 8]. Diverse molecular changes take place in the sequential progression from precursor lesions to PDAC. Researchers have coupled improved understanding of the sequence of molecular changes in the progression from PanIN to PDAC with the establishment of genetically modified animal models that recapitulate these changes [9]. Such molecular alterations provide a road map for understanding PDAC development and progression. Improved diagnostic tools for the detection of preneoplastic lesions and PanINs are urgently needed to effectively control pancreatic cancer. One of the major goals in the biomarker field is to develop noninvasive diagnostic strategies based on these genetic alterations [10]. Thus, molecular biomarkers that improve the efficiency in detection of pancreatic cancer would greatly improve the treatment of PDAC.

Histopathology of PanIN

Morphological and molecular transformation of mutated cells results in the formation of precursor lesions [6]. Precursors of PDAC include PanIN, intraductal papillary mucinous neoplasia, mucinous cystic neoplasia, and intraductal tubular papillary neoplasia, of which PanIN is the most common [11]. Briefly, PanINs are microscopic lesions (<5 mm in diameter) generated in the small pancreatic ducts. The lesions are papillary or flat and composed of columnar and cuboidal cells with various amounts of mucin. According to their progression, PanINs are classified as low-grade (PanIN-1A and PanIN-1B), intermediate-grade (PanIN-2), or high-grade (PanIN-3) PanINs, indicating tissue deterioration into invasive neoplasia. The low-grade PanINs can be flat (PanIN-1A) or papillary (PanIN-1B), characterized by the absence of nuclear atypia and presence of nuclear polarity. PanIN-2 is more complicated than PanIN-1, having more nuclear changes such as loss of nuclear polarity, nuclear crowding, variation in nuclear size (pleomorphism), nuclear hyperchromasia, and nuclear pseudostratification; however, mitosis is rarely observed. PanIN-3, also referred to as pancreatic carcinoma *in situ*, exhibits extensive loss of polarity, nu-

clear atypia, and frequent mitoses. However, as a preinvasive lesion, PanIN-3 still exists in the basement membrane [3, 8]. The incidence of PanIN-1 is associated with age, and it is commonly detected, whereas PanIN-3 is uncommon in individuals with invasive pancreatic cancer.

Molecular Biology of PanIN

Various molecular alterations with major genetic irregularities affecting signaling pathways in each stage of progression from PanIN to PDAC are well characterized. These events destabilize multiple molecular processes, causing further aberrant cell-cycle progression, cell division, and cell growth. Early genetic events in PanIN-1A/B development include mutation of KRAS, epidermal growth factor receptor (EGFR), mucins (MUC1 and MUC6), TFF1, p16INK4A, S100A1, MUC5AC, S100A6, p21WAF1/C1P1, HER-2/neu, prostate stem cell antigen, fascin, matrix metalloproteinase (MMP)-7, and HOXB2 and telomere shortening [2-5, 8]. These mutations are followed by changes in Id-1/Id-2, cyclinD1, cyclooxygenase (COX)-2, 5-lipoxygenase, Hes1, Notch, pepsinigen C, KLF4, HOXA5, GATA5, gastrin, billin1/2, and CRABP1 in PanIN-2 and changes in Tp53, DPC4/Smad4, BRCA2, S100p, SHH, MUC4, TSLC-1, FAP, TopoIIa, 14-3-3s, Ki-67, and FOXM1 in PanIN-3 or PDAC [12, 13-21]. Oncogenic mutations of *KRAS* are detected in more than 90% of invasive pancreatic adenocarcinomas, whereas *BRAF* is mutated in a small subset of pancreatic cancers wild-type for *KRAS* [9, 12, 22]. When KRAS is activated by signaling partners such as EGFR, downstream signaling cascades, including Raf, mitogen-activated protein kinase, and phosphoinositide 3-kinase/AKT, are activated. Consequently, several signaling cascades are also deregulated, including Wnt, EGFR, mitogen-activated protein kinase, phosphoinositide 3-kinase/AKT, transforming growth factor-β, arachidonic acid metabolism, Notch, hedgehog, src, focal adhesion kinase, insulin-like growth factor, hepatocyte growth factor receptor, gastrin, cholescystokinin, and SMAD. Also, angiogenesis may be activated, cancer stem cells (CSCs) may emerge, and shortening of telomeres and other alterations may occur. A model of genetic progression of PanIN is shown in Figure 1, and the detailed molecular aberrations are described below. Molecular pathobiology is the phenotype of pancreaticlesions, often suggesting aberrant molecular changes at the genetic, transcriptomic, epigenetic, and proteomic levels that correlate with histological stages of PanIN development [9, 11-15, 23].
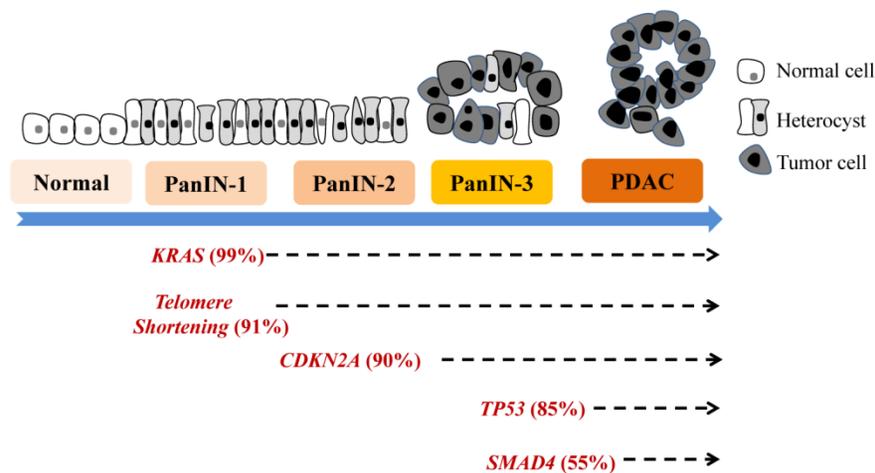
Figure 1. Genetic progression model of pancreatic carcinogenesis. The main molecular alterations that accumulate during pancreatic carcinogenesis can be classified as early (telomere shortening and activating KRAS mutations), intermediate (inactivating mutations or epigenetic silencing of CDKN2A), and late (inactivating mutations of TP53 and SMAD4) genetic events. More new molecular markers are emerging.

Molecular Biomarkers of Early-Stage PanINs

The distinct genetic changes generated in PanIN-1s are telomere shortening and gene mutations, including KRAS, cyclin-dependent kinase (CDK) inhibitor 2A, and p21WAF/CIP1 mutations [9, 12, 22].

Telomeres located at the ends of chromosomes are repeats of short DNA sequences that prevent the chromosomes from fusing during cell division. Researchers have detected remarkable shortening of telomeres in more than 90% of PanIN-1 cases as compared with that in normal ductal epithelium. [24]. Telomere shortening therefore is one of the earliest demonstrable genovariations in pancreatic cancer progression. Telomere shortening results in abnormal fusion of chromosome ends, chromosomal instability, and gene translocation followed by missegregation during mitosis. All of these variations eventually promote neoplastic progression [25]. Intact telomeres may serve as caretakers of the pancreatic ductal genome, and loss of telomere integrity in PanINs sets the stage for progressive accumulation of chromosomal abnormalities and, eventually, generation of frank neoplasia.

KRAS family members encode for small GTP-binding cytoplasmic proteins and regulate cell-cycle progression. KRAS mutation is one of the earliest genetic abnormalities in pancreatic carcinogenesis. The KRAS oncogene (chromosome 12p) is activated by a point mutation in more than 90% of pancreatic cancers, especially in codon 2 [10, 26-28]. Several signaling pathways downstream from KRAS including BRAF/mitogen-activated protein kinase and phosphoinositide 3-kinase/AKT, also may be activated by KRAS mutations [29-31]. However, RAS mutations are fairly common not only in patients with chronic pancreatitis but also in healthy individuals, causing concern about false-positivity in the diagnosis of pancreatic cancer [32, 33]. Inhibition of the KRAS signaling for treatment of pancreatic cancer using farnesyltransferase inhibitors has been disappointing thus far [34-36]. KRAS mutation is therefore a potentially interesting target for early detection and diagnosis of pancreatic cancer.

Progression of PanIN is related to loss of tumor suppressor function. The CDK inhibitor2A/p16 gene, which is located on chromosome arm 9p, encodes for a cell-cycle checkpoint protein and inhibits the cell cycle at the G1/S checkpoint by binding to CDKs, including CDK4 and CDK6 [37]. However, this gene is not expressed in PanIN-1 cells and is inactivated in more than 95% of pancreatic cancer cases [38, 39]. Their homozygous deletion and intragenic mutation in combination with loss of the remaining allele or hypermethylation of the promoter region are the mechanisms of gene silencing in pancreatic carcinogenesis [40, 41]. In some patients, loss of p16 causes familial atypical multiple mole melanoma syndrome, which is highly correlated with an increased risk of pancreatic cancer [24]. In genetically engineered mice, silencing of CDK inhibitor 2A induces the development of undifferentiated carcinoma [41].

p21WAF/CIP1 is a CDK inhibitor that inhibits the activity of cyclin E/cdk2 complexes and prevents phosphorylation of the Rb protein. Unlike proliferation and cell-cycle antigens, p21WAF/CIP1 is overexpressed in PanINs, especially the lower grade forms (PanIN-1A, 16%; PanIN-1B, 32%; PanIN-2, 56%; PanIN-3, 80%), leading to cyclin D1 abnormalities [39]. However, the mechanism of overexpression of

p21WAF/CIP1 is not clear. A reasonable explanation is that overexpressed p21WAF/CIP1 sends negative feedback signals via the ductal epithelium as a result of activation of other mitogenic pathways, such as the KRAS-mediated signaling pathway.

MMP-7 is a member of the MMP family of zinc-dependent extracellular proteases. It takes part in cancer invasion and metastasis and endows resistance to apoptosis to cancer cells [43, 44]. MMP-7 is not expressed in a normal pancreas but is overexpressed in PanINs (>70% of PanIN-1s and the majority of invasive pancreatic adenocarcinomas), which is in line with its proposed tumorigenic characteristics [45]. That is an early event in pancreatic carcinogenesis.

In addition, prostate stem cell antigen is overexpressed in 60% of invasive pancreatic cancers, including about 30% of PanIN-1s, 40% of PanIN-2s, and 60% of PanIN-3s, suggesting the potential for its overexpression as an early event in pancreatic cancer progression models [46]. Also, MUC1 expression in normal pancreatic ducts and acini is responsible for the formation of lumina. However, MUC1 is also often expressed in invasive PDAC cells [47, 48]. Authors reported that MUC1 was expressed in 6% of PanIN-1A and 5% of PanIN-1B cases compared with 43% of PanIN-2 and 85% of PanIN-3 cases [43]. Thus, MUC1 expression in normal intralobular and interlobular ducts appears to be decreased in the low-grade PanINs (PanIN-1A and -1B), but MUC1 appears to be highly expressed in the high-gradePanINs and even invasive adenocarcinomas [12]. In contrast with MUC1, MUC5, the gastric foveolar mucin, is not expressed in normal ductal epithelium, but it is overexpressed in PanINs at all stages [46, 49]. These mucins are also potentially detectable targets in cancer cells and may be developed as therapeutic agents for precursor pancreatic lesions [50].

Molecular Biomarkers of Intermediate- and Late-Stage PanINs

As with gene mutations in the early-stage PanINs, such as KRAS mutations, diverse downstream molecular aberrations, including cyclin D1, COX-2, TP53, and SMAD4 mutations, are identified in the intermediate- and late-stage PanINs,

As a co-factor involved in the phosphorylation and inactivation of Rb protein, cyclin D1 plays a key role in cell-cycle regulation [48]. It is overexpressed in more than 60% of PDAC cases [52, 53]. In addition, overexpression of cyclin D1 is reported to be an intermediate step in carcinogenesis along with its nuclear overexpression in 29% of PanIN-2 cases and 57% of PanIN-3 cases [46]. This has been associated with

poor prognoses and decreased survival durations [54].

COX-2 mediates the metabolism of arachidonic acid into prostaglandins and other proinflammatory products. In tumorigenesis, COX-2 metabolites activate a series of signaling pathways, such as cancer cell proliferation, survival, invasion, and angiogenesis [55]. These processes may be secondary to activation of mitogen-activated protein kinase signaling and nuclear factor κB-mediated signaling [56]. The expression of COX-2 is higher in pancreatic tumors, including PanINs and PDAC, than in normal pancreatic ducts. Also, the expression of COX-2 is markedly higher in PanIN-2/3 than in PanIN-1A/1B [57]. Researchers suggested that selected COX-2 inhibitors are potential targets for chemoprevention of pancreatic cancer [58].

TP53 protein is a transcription factor that modulates molecules primarily involved in cell-cycle arrest and apoptosis [59]. The TP53 gene, located on chromosome 17p, is almost always inactivated by a combination of intragenic mutation and loss of the second wild-type allele in approximately 50-75% of pancreatic cancers [60]. Emerging evidence has demonstrated that loss of p53 function contributes to the genomic instability of pancreatic cancers [61]. Mutation of the TP53 gene is detected by immunolabeling for nuclear accumulation of the p53 protein [62], which can be exploited to assess lesions such as PanINs. Using immunohistochemistry, researchers observed obvious p53 accumulation in advanced PanIN-3s, which is consistent with TP53 gene mutation being a late genetic event in pancreatic cancer progression [46].

SMAD4 (DPC4) protein is critical to the signal transduction cascade that involves transforming growth factor-β and multiple targets in the transforming growth factor-β pathway. SMAD4/DPC4, which is located on chromosome arm 18q, is inactivated by its homozygous deletion and intragenic mutations accompanied by loss of the other allele in 55% of pancreatic cancer cases [63-65]. Recently, authors reported SMAD4 mutations to be associated with poor prognosis for and widespread metastasis of pancreatic cancer [66, 67]. Using nuclear labeling with immunohistochemistry, loss of SMAD4 is generally observed in pancreatic carcinogenesis, such as in PanIN-3s and infiltrating adenocarcinomas [68]. Loss of SMAD4 expression can be regarded as an important genetic event in the development of late PanINs, as well. The BRCA2 gene is part of a class of so-called caretaker genes that play important roles in DNA repair and maintenance of genome integrity. Furthermore, loss of BRCA2 gene function leads to pancreatic carcinogenesis [59]. Germ-line BRCA2

gene mutations occur in about 7-10% of patients with pancreatic cancer, including some with apparently sporadic disease. In one study, among three cases of pancreatic cancer with germ-line mutations of BRCA2, loss of the remaining wild-type allele occurred in only a single PanIN-3 and no low-grade PanINs, suggesting that inactivation of the BRCA2 gene is a late genetic event in pancreatic carcinogenesis [69].

Investigators found that expression of SOX17, a transcription factor that regulates biliary development and differentiation [70], increased throughout progression of PanINs of all stages using immunohistochemistry and tissue microarrays [71]. When combined with Kras^{G12D} expression, SOX17 overexpression enhanced pancreatic tumorigenesis, suggesting that SOX17 expression and the ensuing biliary transdifferentiation promote tumor formation [71].

Furthermore, researchers have observed changes in the expression of many other proteins and apomucins, such as the proliferating antigen topoisomerase IIα, in PanIN and PDAC cases using immunohistochemistry and tissue microarrays. All of these genetic changes in PanINs and PDAC are summarized in Table 1 [16-21, 23, 61].

Table 1. Molecular and genetic abnormalities in PanINs

Abnormality	Normal pancreatic tissue	PanIN-1A	PanIN-1B	PanIN-2	PanIN-3	PDAC
Mucin 1 [112]	+				+	+
Kras mutation [113]		+	+	+	+	+
Telomere shortening [21]		+	+	+	+	+
PSCA [114]		+	+	+	+	+
Mucin 5 [115]		+	+	+	+	+
Fascin [116]		+	+	+	+	+
MMP-7 [117]		+	+	+	+	+
SOX17 [118]	+	+	+	+	+	+
P16 [119]				+	+	+
Cyclin D1 [120]				+	+	+
KLF4 [121]			+	+	+	+
TP53 [122,123]					+	+
SMAD4 [124]					+	+
Topoisomerase IIα [115]					+	+
Ki-67 [125]					+	+
14-3-3σ [115,126]					+	+
Mucin 4 [127]					+	+
FOXM1 [128,129]					+	+
Mesothelin [130]						+
Glypican-1 [4]						+

PSCA, prostate stem cell antigen.

Misregulation of MIcroRNA Expression in PanINs

Besides the molecular changes that occur at the DNA and protein level during progression from PanIN to PDAC, molecular events at the RNA level, such as microRNA (miRNA) changes, are identified during pancreatic carcinogenesis.

MiRNAs are small noncoding RNAs of about 22 nucleotides that primarily bind to the 3' untranslated region of target mRNA and then negatively regulate protein expression at the posttranscriptional level by inhibiting translation and/or degrading target mRNA [69]. Researchers have identified more than 400 miRNAs in the human genome, many of which are implicated to have roles in regulation of cellular differentiation, proliferation, and apoptosis [37]. They are reported to be closely correlated with the progression of many diseases, including cancer [73]. Several miRNAs, including miR-21, miR-34, miR-146a, miR-155, miR-196a-2, and miR-200a/b, are overexpressed in PDAC cells [5, 16, 20, 21, 24-28], and some of them are aberrantly expressed in PanINs. For example, miR-155 overexpression is evident in PanIN-2s, and aberrant miR-21 expression is evident in PanIN-3s. Therefore, misregulation of miRNA expression is an early event in PanIN development [74]. With regard to alterations at the transcriptomic level, researchers recently exploited miRNAs as potential markers and therapeutic targets for pancreatic cancer.

In addition, miR-494 is an important regulator of pancreatic cancer progression. In a recent study, we found that loss of SMAD4 expression in PDAC cells leads to reduced levels of miR-494 expression, increased levels of FOXM1 expression, and nuclear localization of β-catenin, meaning that miR-494 may be developed as a prognostic marker or therapeutic target for PDAC [17]. Extensive cross-talk between FOXM1 signaling and other major signaling pathways further substantiates a pivotal role for FOXM1 in pancreatic cancer development and progression (Figure 2).
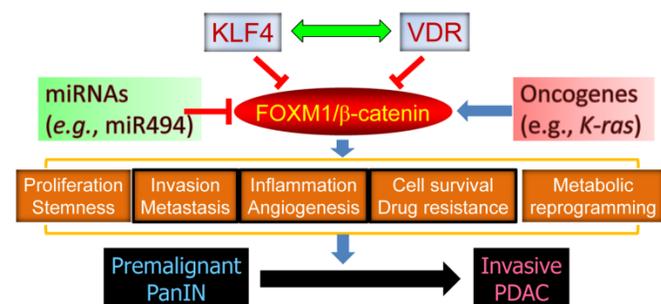

Figure 2. The central role of FOXM1 signaling in pancreatic carcinogenesis. FOXM1 regulates various aspects of cancer cell biology via regulation of its downstream target genes and drives the progression of premalignant PanIN to invasive PDAC. Gain-of-function mutation of oncogenes and loss-of-function mutation of tumor suppressors may activate FOXM1 expression and signaling, and KLF4, VDR, and miR494 are known to repress the expression and function of FOXM1/β-catenin signaling.

Strategies for Therapeutic Intervention for PanINs

Early diagnosis of pancreatic cancer would enable the use of chemoprevention strategies to impede progression from PanIN to invasive carcinoma [22]. However, detection of pancreatic cancer at an early stage is difficult using the currently available modalities. The challenge in early diagnosis of pancreatic cancer is detection of early emerging symptoms. Extensive investigations of molecular signaling in pancreatic cancer cells have increased understanding of the molecular genetics of progression from PanIN to PDAC and resulted in progress in the diagnosis, staging, and treatment of localized pancreatic cancer. However, those studies did not result in any significant breakthroughs in early detection or significantly improve prognoses. Therefore, novel therapeutic strategies based on the molecular changes described above and on the concept of CSCs and stromal targeting should be explored to improve early diagnosis of and therapy for pancreatic cancer.

KRAS

KRAS is mutated in pancreatic cells early in the development of pancreatic cancer as evidenced by its identification in PanINs [75]. KRAS1 is a pseudogene, whereas KRAS2 is the proto-oncogene that results in a dominant-active form of the KRAS GTPase [26, 29]. KRAS2 is commonly mutated in many cancer types, including more than 90% of pancreatic cancer cases. The genetically modified model of pancreatic cancer established by Collins et al. [76] facilitated controllable activation and inactivation of oncogenic KRAS under different experimental conditions, including caerulein-induced pancreatitis and mutation of p53, which accelerated cancer onset. Of major importance to potential treatment of pancreatic cancer is that the oncoprotein KRAS is required for not only the initiation but also the maintenance of the disease in mice [76]. However, an important point is that KRAS mutations are fairly common, not only in patients with chronic pancreatitis, a recognized risk factor for pancreatic cancer [77], but also in healthy individuals [78]. These facts underline the fact that additional downstream genetic hits are urgently needed for accurate detection of pancreatic cancer based on KRAS mutations [79].

Interestingly, antroquinonol, a new Kras-targeting inhibitor, has had a marked anticancer effect on human pancreatic carcinoma cells, inducing cross-talk among apoptosis, autophagy, and senescence [80-82].

Tyrosine kinase receptors

Researchers have made considerable efforts in development of a number of tyrosine kinase receptors as potential therapeutic targets for pancreatic cancer, including EGFR, a member of the HER2 family of receptor tyrosine kinases, and insulin-like growth factor receptor. The National Cancer Institute of Canada Clinical Trials Group PA.3 phase III study (n = 569) was the first study to demonstrate a survival benefit of combination therapy with a tyrosine kinase inhibitor and gemcitabine in patients with pancreatic cancer [83]. Specifically, the combination of gemcitabine and the oral EGFR tyrosine kinase inhibitor erlotinib improved the median survival duration by 2 weeks over that with gemcitabine alone in patients with advanced pancreatic cancer (6.4 months versus 6.0 months). Overexpression of core genes in the EGFR pathway, such as AKT3 and PIK3CA, was predictive of response to treatment with EGFR inhibitors in an additional cohort of eight patients with implanted PDACs [84]. These findings demonstrated that treatment with tyrosine kinase inhibitors is promising in patients with PanINs and PDAC.

Investigators have successfully used two pharmacological approaches to inhibition of EGFR function in cancer treatment: delivery of neutralizing monoclonal antibodies and small molecule tyrosine inhibitors. The results of randomized trials of the addition of EGFR-targeted agents to gemcitabine compared with gemcitabine alone have been disappointing, although results of treatment with the EGFR tyrosine kinase inhibitor erlotinib were statistically significantly better but of marginal clinical benefit [85]. Recent studies demonstrated that targeting EGFR can be used for early diagnosis of orthotopic pancreatic tumors *in vivo* [86, 87].

Notch

The Notch signaling pathway makes cell-fate decisions during embryogenesis. Notch signaling is produced after cell-to-cell contact and initiates the engagement of Notch receptors by Notch ligands [88]. The binding of Notch receptors by Notch ligands causes extracellular proteolysis via the action of metalloproteinase, a tumor necrosis factor-converting enzyme, ultimately resulting in γ−secretase–dependent intracellular proteolysis and release of the active notch intracellular domain from the plasma membrane. This domain is subsequently translocated to the nucleus and initiates the transcriptional activation and repression of target genes. The activity of Notch varies in different kinds of cancer cells, having oncogenic activity in some but tumor-suppressive activity in others [88]. Notch also is an oncogene involved in the progression of pancreatic cancer. Notch is a downstream pathway of KRAS, EGFR, and transforming growth factor-α signaling in pancreatic

carcinogenesis that promotes cancer vascularization. As reported previously, aberrant regulation of the Notch pathway in pancreatic cancer cells contributes to tumor initiation, progression, and maintenance, and researchers have made efforts to target this pathway for cancer therapy [89]. Downregulation of Notch expression by small interfering RNA or curcumin is capable of inhibiting cell growth and inducing apoptosis in pancreatic cancer cells *in vitro* [90]. Therefore, regulation of the Notch pathway is a potential method of pancreatic cancer therapy. However, more investigations using *in vitro* and *in vivo* pancreatic cancer models should be carried out.

Authors reported that the Notch-targeting inhibitor RO4929097, an oral inhibitor of the γ-secretase enzyme, is safe when given as a single agent in patients with advanced solid pancreatic tumors [91]. Notch-1 and Notch-4 are novel transcriptional targets of PEA3 in breast cancer cells, and targeting of PEA3 and/or Notch pathways may be a new therapeutic strategy for triple-negative and possibly other subtypes of breast cancer [92, 93].

BRCA2 poly(ADP-ribose) polymerase inhibition

As a tumor suppressor gene, BRCA2 takes part in DNA damage repair, and germ-line mutations of the BRCA2 gene increase the risk of pancreatic cancer [90]. These findings demonstrated that inactivation of the BRCA2 gene is responsible for approximately 10% of familial pancreatic cancer cases [90, 94]. Therefore, activation of the BRCA2 gene can be expected to prevent the development of pancreatic cancer. Patients with pancreatic cancer have received treatment with poly(ADP-ribose) polymerase inhibitors, which target BRCA-deficient cancers and have exhibited exciting effectiveness [95]. Several clinical-stage poly(ADP-ribose) polymerase inhibitors, including veliparib, rucaparib, olaparib, niraparib, and talazoparib, have undergone evaluation of their poly(ADP-ribose) polymerase-trapping activity and use in pancreatic cancer therapy [93-95].

CSCs

Much interest in pancreatic cancer research has focused on CSCs. Nguyen et al. [99] defined CSCs as cells in a malignant clonal population that can propagate cancer. CSCs are believed to be responsible for self-renewal, maintenance, and metastasis of tumors that must be eradicated for cancer therapy. Although traditional therapies are capable of eliminating rapidly proliferating cells within the tumor bulk, CSCs are thought to be somewhat quiescent and may evade treatment. Thus, strategies for targeting these cells are expected to result in successful cancer treatment. Li et

al. [100] identified c-Met as a stem cell marker in pancreatic cancer cases and reported that expression of c-Met was necessary for tumor growth and metastasis. Therefore, targeting pancreatic CSCs via c-Met and related pathways is a promising platform for stopping the development of tumor metastases [100]. In a recent study, investigators found that DCLK1 marked a morphologically distinct subpopulation of cells with CSC properties in preinvasive pancreatic cancer cases [101]. Kure et al. [102] examined the correlation between development of PanIN and expression of CSC markers, including CD24, CD44, CD133, CXCR4, ESA, and nestin, using immunohistochemical analysis and detected CD24-, CD44-, CXCR4-, ESA-, and nestin-positive cells in the following tissues, which are listed in order of increasing percentage: normal duct < low-grade PanIN < high-grade PanIN < PDAC.

Stromal targeting therapy

Many anticancer drugs have been highly effective in cell models and xenograft and/or allograft mice models of pancreatic cancer but have failed in patients with pancreatic cancer. An emerging explanation for the discrepancy between their effectiveness in preclinical and clinical trials is the absence of stromal microenvironments in pancreatic cancer cells and mouse models. A pancreatic tumor is characterized by a dense fibrotic stromal matrix [103] composed of activated fibroblasts/stellate cells, inflammatory cells, and other cell types, such as endothelial cells. These cells form a microenvironment that plays a crucial role in cancer initiation, progression, and chemoresistance [104-106]. The delivery of chemotherapeutic drugs to cancer cells in the stroma *in vivo* is the first step in cancer cell internalization and targeting. Investigations using genetically engineered mice with pancreatic cancer bearing well-developed stromal components similar to those of human tumors have indicated that drugs do not penetrate the stromal mass and hence are not able to effectively approach cancer cells [103, 106]. Therefore, development of approaches to modifying the pancreatic tumor stroma, which facilitates the access of chemotherapeutic drugs to cancer cells, should attract extensive attention. Marimastat is a broad-spectrum synthetic MMP inhibitor that researchers first tested in a large randomized phase 3 trial in patients with advanced pancreatic cancer [107-109]. *In vivo* studies demonstrated that human pancreatic stellate cells in conditioned medium increased pancreatic tumor cell proliferation, migration, invasion, and colony formation and that treatment with human pancreatic stellate cells in conditioned medium rendered pancreatic cancer cells more resistant to gemcitabine and radiation therapy

[110, 111].

Conclusions and Future Directions

A series of molecular genetic alterations, such as KRAS mutations, occur in PanIN, the most common precursor of PDAC. These molecular alterations are closely correlated with each stage in the progression from PanINs to PDAC by affecting downstream signaling pathways. A lack of early detection of and effective interventions for these genetic irregularities is a major contributor to the poor prognosis and dismal survival rates for pancreatic cancer. The availability of noninvasive molecular biomarkers of PanIN provides the basis for designing rational early-detection strategies and therapeutic intervention trials for pancreatic cancer. Use of these markers can resolve the diagnostic conundrum for early detection of PanIN progression to invasive carcinoma and serves as a guideline for the design of novel therapeutic intervention strategies. Chemical entities and therapeutic genes such as small interfering RNA and miRNA may be employed to regulate signal pathways, including KRAS, tyrosine kinase receptors, Notch, and BRCA2, for pancreatic cancer therapy. In addition, targeting inhibition of CSCs and regulation of tumor stroma are emerging promising routes. Further understanding of the detailed downstream cascades of molecular genetic alterations and development of corresponding targeting drugs and therapeutic strategies in to block the stepwise progression of PanIN are needed. Finally, it is important to use some animal models other than clinical patient cohorts for discovering early diagnostic markers, *e.g.*, GEMMs, since most of the clinical patients already develop pancreatic cancer thus some of the early events may be missed. Moreover, it will be convenient to collect precancerous tissue and blood specimens during the monitoring of the animal models.

Acknowledgements

Competing Interests

The authors have declared that no competing interest exists.

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