Metastatic pancreatic cancer: Mechanisms and detection (Review)

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Abstract. Pancreatic cancer (PC) is a lethal malignancy. Its prevalence rate remains low but continues to grow each year. Among all stages of PC, metastatic PC is defined as late-stage (stage IV) PC and has an even higher fatality rate. Patients with PC do not have any specific clinical manifestations. Most cases are inoperable at the time-point of diagnosis. Prognosis is also poor even with curative-intent surgery. Complications during surgery, postoperative pancreatic fistula and recurrence with metastatic foci make the management of metastatic PC difficult. While extensive efforts were made to improve survival outcomes, further elucidation of the molecular mechanisms of metastasis poses a formidable challenge. The present review provided an overview of the mechanisms of metastatic PC, summarizing currently known signaling pathways (e.g. epithelial-mesenchymal transition, NF-κB and KRAS), imaging that may be utilized for early detection and biomarkers (e.g. carbohydrate antigen 19-9, prostate cancer-associated transcript-1, F-box/LRR-repeat protein 7 and tumor stroma), giving insight into promising therapeutic targets.

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1. Introduction

Pancreatic cancer (PC) is the seventh leading cause of cancer-associated mortality worldwide (1) and it is predicted to become the second leading cause of cancer-related death in Western countries in the next decades (2). An up-to-date report estimated 57,600 new cases of PC in 2020. Among all cancer types, the 5-year survival rate is the lowest for all stages of cancers of the pancreas combined (9%) (3). The 5-year overall survival (OS) for metastatic PC is even poorer, namely as low as 2% (4,5), with a median survival expectancy of <1 year with current treatments (2). PC is hyperaggressive and evolves from non-invasive precursor lesions. Therefore, only minor symptoms may be noticed in the early stage. The lack of specific risk factors makes early detection a formidable challenge. The tumor grows along with genetic and epigenetic alterations. Delays in diagnosis lead to poor prognosis. Furthermore, no consensus has been reached regarding the optimal therapy. Focusing on early detection of metastatic PC at least prolongs the survival, improves life quality and reduces treatment-associated toxicities.

Metastatic PC is defined as stage IV cancer and it is not possible to completely remove it by surgery. Recent research has shifted the focus on prevention and early detection. However, effective and feasible screening strategies may provide accurate identification of at-risk individuals. Extensive studies have managed to determine signaling pathways, biomarkers or their combinations that may be accurate in detecting PC or predicting tumor metastasis (4,5). The intricate communication among these elements and the low prevalence of PC make such attempts challenging.

2. Detection methods

Imaging modalities: CT and MRI. At diagnosis, it is common to detect that PC has already metastasized to a certain extent, so that curative surgery is impossible. Tumor cell migration has been a long-standing obstacle for disease management. At first, the tumor spreads confined to the pancreas. Subsequently, it spreads to adjacent organs, blood vessels that surround the pancreas or to other parts, but still within the abdomen. Much more aggressive types spread to distant organs such as the liver, lungs or bones (6). While it is rarely observed, it may also spread to the brain (7). Tumor cells travel to other body regions through the blood or lymphatic system. Therefore, the

early identification of signs of such migration is paramount to the treatment course and improve OS outcomes. Accurate staging of PC is essential for the course of therapy. The diagnosis of PC is made based on pathological results that are combined with imaging. Commonly used imaging modalities for tumor assessment include computed tomography (8), magnetic resonance imaging (MRI), endoscopic ultrasound (EUS), positron emission tomography (PET) and magnetic resonance cholangiopancreatography. Furthermore, advanced techniques such as cinematic rendering and radiomics may be applied. Among all of those imaging techniques, CT and MRI are first-line diagnostic modalities for suspected PC. Both of them are capable of evaluating resectability. While most cases of PC have metastasis at the time-point of diagnosis, early detection of PC metastases via imaging features is still feasible.

CT is inarguably the optimal imaging modality in the initial assessment (9-11). CT uses X-rays to create a cross-section image of a region of the body. It accurately assesses metastatic spread or provides clues to treatment strategies for borderline resectable tumors (12). Rapid imaging with good spatial and temporal resolution makes CT a widely-used technique to assist diagnosis (13,14). CT is preferred over MRI due to its lower cost and widespread availability (15). CT is also one of the two imaging modalities (another one is ultrasound) that is able to detect PC metastases (16). However, this is debatable. A study concluded that the performance of CT is poor in the diagnosis of small hepatic metastases (17). In addition, it is useful in quantifying changes in abdominal fat and lumbar muscle mass with the emergence of PC, thus helping with early detection (18,19). A recent study determined that pretreatment CT quantitative imaging biomarkers from texture analysis and tumor size combined may predict survival outcomes compared with imaging biomarkers alone (20).

The use of CT has undergone a revolution. A large number of studies have applied different combinations of CT to determine whether this improves the accuracy and sensitivity. PET/CT is an advanced technique. It provides 3-dimensional imaging based on the detection of radiation from the emission of positrons. PET/CT helps with observing molecular activities and is thus promising in the early detection of cancers and prediction of treatment response. It is inarguably of prognostic value in patients with PC, regardless of metastases (21-23). A study evaluated the application of PET/CT for presurgical tumor staging of PC and concluded that its utility is rather limited (24). Hu *et al* (25) examined 19 patients with metastatic PC and determined that ^{18}FDG-PET/CT is useful in the early detection of metastases. A meta-analysis evaluated the sensitivity and accuracy of CT, ^{18}FDG-PET and ^{18}FDG-PET/CT, respectively, analyzing 11 eligible articles and 5 types of cancers (head and neck cancer, lung cancer, melanoma, sarcomas and colorectal cancer). The study determined that the integration of CT with PET performed best in assessing distant metastases (26). However, PC was not included in this analysis, possibly due to its low prevalence. It may be possible to perform another meta-analysis for PC metastases with the accumulation of cases in the near future. Furthermore, a slightly modified version of PET/CT, namely PET/contrast-enhanced (CE)-CT, was reported to have a diagnostic accuracy rate of 80% in the evaluation of staging in resectable PC, and surprisingly, it was as high as 94% for distant metastasis of PC, while it was low for lymph node (LN) metastasis (only 42%) (27).

Multidetector CT is the most widely available and validated imaging modality for staging and diagnosis of PC (28). Thin-section arterial and venous phase imaging allows evaluation of distant metastases (17,29) but requires a timely re-examination within a short period of time (30).

MRI, on the other hand, provided additional information on the stage or presence of small liver metastases that may otherwise be missed on CT. It is used when CT imaging lacks clarity or when CT is not applicable for patients. MRI has a slightly higher sensitivity compared with CT (83-94 vs. 76-96%, respectively) (31-33). The reported accuracy in determining tumor resectability ranges from 73 to 87% for CT and from 70 to 80% for MRI (33). In terms of pancreatic surveillance for individuals at high risk, MRI is preferred due to its higher accuracy in detecting sub-centimeter pancreatic cysts and low ionizing radiation (34-36).

Mizumoto *et al* (37) retrospectively reviewed patients with PC. They indicated that iso- or hypo-attenuating regions were related to regional LN metastasis. Dilation of the main pancreatic duct and other non-enhancement features on preoperative MRI were reported to be helpful in predicting LN metastases. Leng *et al* (38) proposed the predictive value of LN metastases for postoperative recurrence in patients with invasive and noninvasive intraductal papillary mucinous neoplasms (IPMNs), suggesting that secondary signs of tumors may also be predictive factors. A retrospective study analyzed discriminatory signs on CT for patients with chronic calcifying pancreatitis and eventually developed PC. It was concluded that a hypodense mass at diagnosis is a predictive factor (39). As mentioned above, combinations of CT modalities have improved its efficacy to a large extent. Likewise, combining diffusion-weighted MRI with CE-MRI has nearly doubled the early detection of liver metastasis (from 156 to 397 cases) (40).

Machine learning, not surprisingly, paves a road for the automatic extraction of imaging features to classify and discriminate. Unsupervised training to 'read' scans effectively identifies foci that may otherwise be easily ignored or wrongly interpreted by radiologists. Automatic identification of the pancreas achieved high accuracy, with a reported Dice-Sørensen coefficient ranging from 71 to 82% with CT data (41,42), and up to 83% with MR data (43,44).

Apart from all of these merits mentioned above, a standardized reporting template for radiologists is required. Previously, the Society of Abdominal Radiology and the American Pancreatic Association adopted one template, emphasizing the importance of a complete, accurate and reproducible radiology report together with high-quality imaging. A repeat workup with tailored pancreas protocol multidetector CT angiography is beneficial for ruling out the possibility of a tumor (45). Whether early detection may achieve high accuracy and sensitivity through machine learning warrants evaluation of mass data and accurate algorithms. Furthermore, the smart utilization of imaging modalities to screen at-risk individuals should be promoted. A reported 3 and 20% of CT and MRI scans identified pancreatic cysts that are likely to develop into PC (46,47),

emphasizing the importance of a thorough interpretation of scanning images during screening.

Liquid biopsy. Tissue biopsy is the gold standard for the diagnosis of primary or metastatic diseases, but it is invasive. Liquid biopsy, a technique that has been used in lung cancer and breast cancer, is of high diagnostic value. Its clinical diagnostic value has now also been demonstrated for PC, including high-risk cohort surveillance, disease staging and longitudinal monitoring of tumor evolution and progression in response to treatment, as well as analyses to provide genomic and molecular information on potential pancreatic ductual adenocarcinoma (PDAC) (48). Liquid biopsies are minimally invasive and have an improved ability to represent tumor heterogeneity and nonsolid biological tissue, including circulating tumor cells (CTCs), primarily circulating tumor DNA (ctDNA) and exosomes secreted by cancer or normal cells, from all tumor sources, including metastatic sites (49).

CTCs are cells derived from primary and secondary tumors that may enter the vascular system at an early stage and seed to distant organs (50). The content is determined by enrichment and a combination of multiple detection methods (51). CTC has an important role in the transfer cascade reaction, but its limitations are low sensitivity, rarity and high heterogeneity (49). The number of CTCs may vary over time and space, with blood passing through the portal vein to the liver immediately after leaving the pancreas and large CTCs and clusters may become trapped. Furthermore, the blood flow in pancreatic malignancies is 60% less than that in normal pancreatic tissue, so that peripheral blood CTCs may not be the best choice for diagnosis and prognosis of PC, but portal vein samples may be more representative of the CTC population (48). CTC detection tends to increase with tumor staging and is useful for the diagnosis of PC, but does not provide any relevant prognostic information (51).

ctDNA, also known as prenatal cell-free DNA, is the DNA released into the plasma by CTCs, the primary tumor or secondary tumor depositing necrotic or apoptotic cells as a result of cell death, but it is difficult to obtain effectively and is able to reflect the tumor load of patients with solid pancreatic tumor (49-51). The specificity of ctDNA was reported to be much higher than that of CTC and its sensitivity was slightly lower than that of CTC (49). CtDNA detection is based on KRAS mutations, but KRAS mutations are present not only in PDAC, but also in various other types of malignancies and even in chronic pancreatitis (CP) (50). Therefore, its relatively low specificity should be considered. The detection of ctDNA requires ultra-sensitive techniques and a large amount of plasma (12). At present, there is no reliable clinical evidence for its role in detecting early cancer.

Exosomes are small vesicles released from the plasma membrane by almost all cells, including cancer cells (50). Exosomes provide substrates for molecular profiling of circulating nucleic acids (such as exosomal DNA and exosomal RNA) and may also transfer a variety of biologically active molecules [such as proteins, lipids and pathogenic microRNAs (miRNAs/miRs) or mRNAs] from donor cells to recipient cells (48). Pancreatic cells have a strong exocrine function, leading to a high content of exosomes in peripheral blood with easy detection and high sensitivity (49). Since it enters the circulation at an early stage of cancer development, it may be used as a biomarker for early disease detection and tumor surveillance. Ariston Gabriel *et al* (52) illustrated that exosomes act as a carrier of miRNAs and other markers, including miR-196a, miR-1246, miR-191, miR-21, miR-451a, miR-16a and miR-196a, carbohydrate antigen 19-9 (CA19-9), miR-483-3p, miR-1246, miR-4644, miR-4525, miR-451a, miR-21, miR-155, miR196a, miR-1246, miR-4644, miR-3976, miR-4306, CD44v6, Tspan8, EpCAM, MET, CD104, exmiR-21, miR-17-5p, miR-10b, miR-550, miR-10b, miR-21, miR-30c and miR-181a, as well as low miR-let7a, which may be employed as diagnostic markers for PC.

The role of liquid biopsy in the early diagnosis of PDAC is theoretically promising as a standard of care for early diagnosis, molecular stratification, prognosis and predictive utility of PC, and for longitudinal monitoring of the effect of treatment of established disease (48). However, the data available so far appear contradictory and the true role of certain factors remains to be elucidated. One of the major limitations is the lack of standardized testing methods (50). Current technologies are frequently time-consuming, inherently limited in terms of processing and analysis, labor-intensive and potentially costly (48). Therefore, large-scale validation studies are required prior to clinical application (50).

Assay for transposase-accessible chromatin (ATAC)-array. ATAC sequencing (ATAC-seq) uses an overactive TN5 transposon to assess DNA accessibility, simultaneously cleaves DNA and inserts sequencing splices, preferentially in open chromatin regions. DNA sequencing libraries rich in DNA super-accessible regions are being generated and subjected to high-throughput sequencing. The readings are then aligned with the assembled genome to identify areas marked by high-density aligned reads. ATAC-seq, similar to other methods of chromatin accessibility analysis, provides a static assessment of the chromatin structure and reveals local and super-accessible regions. This method has proved to be valuable for high-throughput identification of active cis regulatory elements in a variety of cell types.

To simplify the assessment of chromatin accessibility signatures to the point of clinical utility, Dhara *et al* (53) developed a microarray approach termed 'ATAC-array', where the accessible regions from the differential chromatin accessibility signatures were arrayed on glass slides and then hybridized with fluorescent-labeled ATAC libraries. Applying this method to the original ATAC-seq library and the patient-derived organ-like independent library, they determined the characteristics of chromatin accessibility and transcription factors (TF), such as ZKSCAN1 and HNF1b, which are significantly related to the prognosis of PDAC, providing a novel chromatin-based prognostic paradigm for accurate oncological practice. The ATAC-array technique may be combined with nuclear localization of HNF1B by immunohistochemistry, which provides a simple and achievable prediction of the beneficial and detrimental epigenetic status of the disease for clinical work. However, whether poor patterns of chromatin accessibility contribute to the selection of patients with PDAC for epigenetic 'reprogramming' therapy remains to be determined.

3. Signaling pathways

A deep understanding of the mechanisms of tumor metastases is paramount for disease management. Over the years, scientific researchers have made efforts to discover signaling pathways that may explain the pattern of metastasis during tumor progression. The goal itself is laudable but difficult to achieve and progress is slow. In the present review, it is not possible to cover all of the known signaling pathways. Instead, it was decided to focus on epithelial-mesenchymal transition (EMT), a mechanism that has received extensive attention from researchers, in addition to TANK binding kinase-1 (TBK1)-nuclear factor κB (NF-κB), an emerging signaling pathway that may be a potential therapeutic target candidate, as well as KRAS, as the major contributors of PC. Various relevant signaling pathways are illustrated in Fig. 1.

EMT. EMT is a physiological process that allows cancer cells to undergo morphological and genetic changes through epithelial-mesenchymal transformation, which underlies the highly metastatic function of cancer cells and contributes to their invasion and drug resistance. EMT is triggered by a variety of tumor microenvironmental factors, including cytokines, growth factors and chemotherapy drugs (54). EMT is a potential biomarker for early prognosis, so it is necessary to determine the effect of EMT on the metastatic process of cancer cells. Sannino *et al* (55) identified that BCL9L is a key modulator for invasion and metastasis of PC cells and reduced BCL9L expression delayed the response to TGF-β-induced EMT, which was associated with the loss of proliferation, migration and invasion of PC cells. In a study by Ye and Weinberg (56), TGF-β-induced E-cadherin expression in control Panc-1 cells was significantly down-regulated and accompanied by the expression of mesenchymal genes (SNAI2, VIM) in PC cells, although not significantly. However, this EMT response was significantly reduced in cells that did not express the BCL9L gene (55,57). When the expression level of E-cadherin increased, the expression level of the mesenchymal gene SNA12 decreased. Therefore, the expression of the BCL9L gene has a decisive role in inhibiting PC cells undergoing EMT *in vivo* and may effectively counteract PDAC invasion and metastasis by triggering EMT in addition to the classical WNT signaling pathway (55).

TBK1-NF-κB. The NF-κB family functions as regulators of cell proliferation, differentiation, immune responses, inflammation, invasion and metastasis. The activation of NF-κB is determined by proinflammatory cytokine paracrine loops (58,59). Among those cytokines, IL-1α was indicated to activate NF-κB in metastatic PC cell lines, which in turn induced invasion mainly of the liver. NF-κB directly regulates EMT-TFs. In line with this, suppression of NF-κB by dehydroxymethylepoxyquinomicin degraded EMT-TF expression in PC (60). TBK1 modulates inflammatory signaling cascades and autophagy. Although the number of relevant studies is low, Labelle *et al* (61) reported that when NF-κB is activated synergistically by platelet-derived TGF-β and direct platelet-tumor cell contact, it transforms the cells into an aggressive phenotype and enhances metastasis *in vivo*. Inhibiting the activation of NF-κB and the expression of TGF-β may effectively reduce tumor metastasis. Therefore, the metastasis of tumor cells is mainly through signals derived from platelets outside the tumor *in vivo*. However, this experiment was performed in mouse models of colon cancer and breast cancer and thus, numerous experiments are still required to verify this conclusion (Fig. 1).

KRAS. KRAS is one of the four major driver genes in PC. KRAS protein is a small GTPase *per se*. The functions of KRAS include endocytosis/exocytosis, survival, proliferation, invasion and transformation. When bound to GTP, KRAS is activated. KRAS protein interacts with >80 downstream effector proteins and signaling pathways, such as PI3K-AKT-mTOR, MAPK-MAPK kinase (MEK) or rapidly accelerated fibrosarcoma-MEK-ERK (51). Nuclear TFs are also activated (such as ELK, JUN and MYC), leading to stimulation of cell differentiation, proliferation, migration, transformation, adhesion and survival.

KRAS mutation is an early event in tumorigenesis. An activating point mutation of KRAS of oncogene on codon 12 (exon 2) is observed in the majority of PC cases. The existence of a KRAS mutation predicts poor prognosis in PDAC. Mutated KRAS contributes to tumor growth and metastasis in several ways. First, oncogenic KRAS secrete chemokines to activate T cells, B cells and macrophages, which drive the inflammatory response and tumor growth. Furthermore, the Warburg effect was observed during tumorigenesis, with an increase in glucose uptake and a shift from mitochondrial oxidative phosphorylation to aerobic glycolysis. Finally, high levels of lactate and reactive oxygen species are produced as a result of KRAS mutation (62).

Given that the oncogenic point mutation of KRAS is a frequent event during PDAC, the identification of this mutation in biological fluids and tumor tissues may prove useful in the diagnosis as well as in the prognostic evaluation and therapeutic decision-making. In the past 20 years, evaluation of KRAS mutation testing in patients with PDAC has been discussed in depth. A meta-analysis of mutated KRAS detection in pancreatic juice reported a pooled sensitivity of 59% (95% CI: 54-64%) and a specificity of 87% (95% CI: 84-89%) (63). In addition to pancreatic juice, these studies have involved EUS-fine-needle aspiration samples and, more recently, circulating cell-free tumour DNA. The latter is part of the concept of liquid biopsy that also involves the search for CTCs, exosomes or miRNA. Various test methods are being performed on these samples to detect gene mutations, among which PCR is popular. However, the accuracy of PCR is challenged by poor specimens or complex cellular background. The adoption of digital PCR is complimentary, achieving high sensitivity in the presence of a noisy background (64).

4. Biomarkers

Despite extensive efforts to explore specific biomarkers of cancers, the origins of tumor metastasis have remained to be fully elucidated. It is hypothesized that the metastatic cascade results from an epigenetically altered transcriptional output of the oncogenic signals (65). While a vast number of hypotheses have been postulated, the present review focuses

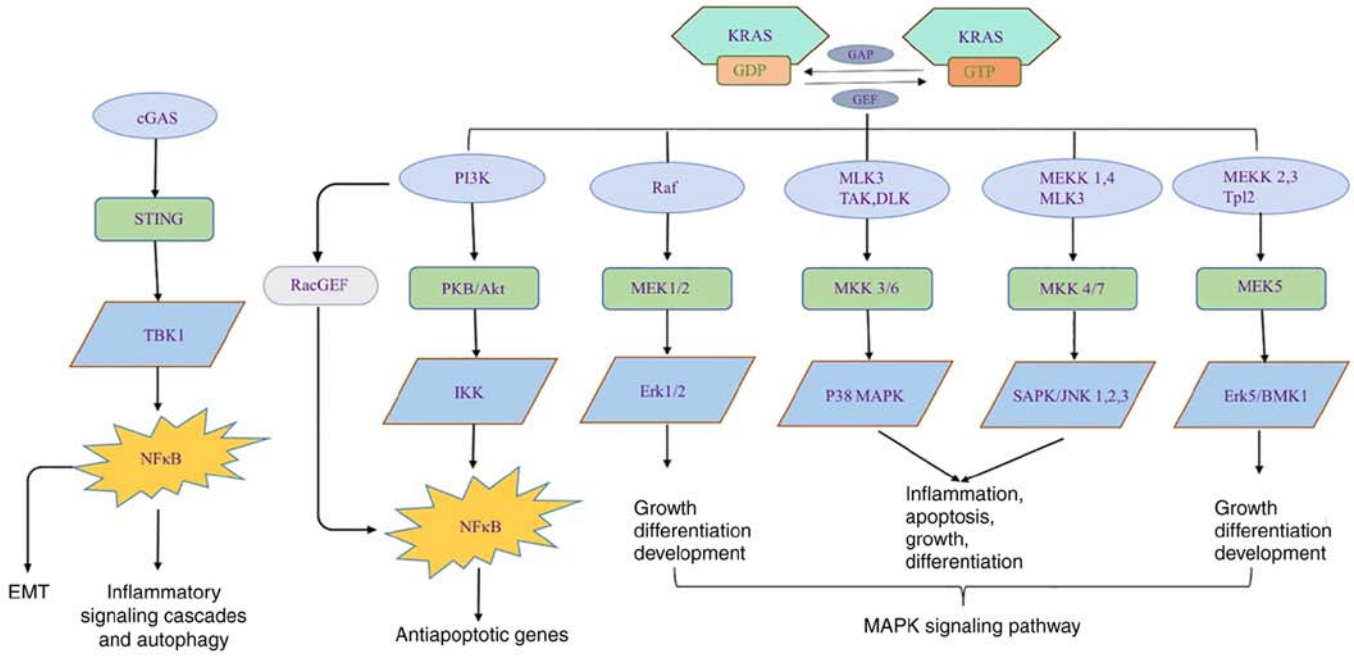

Figure 1. Major signaling pathways in metastatic pancreatic cancer. EMT, epithelial-mesenchymal transition; NF-κB, nuclear factor-κ-light-chain-enhancer activated B cells; GDP, guanosine diphosphate; GTP, guanosine triphosphate; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; TBK1, TANK binding kinase-1; PI3K, phosphatidylinositol 3-kinase; IKK, inhibitor of NF-κB kinase; MEK, MAPK/ERK kinase; Erk, extracellular signal-regulated protein kinase; MLK3, mixed lineage kinase 3; TAK, transforming growth factor β-activated kinase; DLK, dual-leucine zipper kinase; P38 MAPK, P38 mitogen-activated protein kinase; MKK, MAP kinase kinase; SAPK/JNK, stress-activated protein kinase/c-Jun N-terminal kinase; MEKK, MEK kinase; BMK1, big MAP kinase 1.

on 7 biomarkers that were determined to regulate tumor cell migration in PC. These are neutrophil extracellular traps (NETs), prostate cancer-associated transcript-1 (PCAT-1), F-box/LRR-repeat protein 7 (FBXL7), CA19-9, pentraxin 3 (PTX3), tumor stroma and non-coding RNAs.

NETs. Since the discovery of NETs, their role has been widely debated. NETs have beneficial physiological consequences by strengthening the host defense. However, uncontrolled NETs are destructive and associated with cancer metastasis (66). Thus, the function of NETs in tumor progression may always heat a discussion. It was reported that chloroquine (67) represses NETs (67) and slows cancer progression (66). Murthy *et al* (68) further demonstrated the role of CQ in impeding NET formation. In their model, the severity of acute pancreatitis was decreased by CQ, thus improving survival by inhibiting NETs. *In vivo* culture of neutrophils performed by Hiroki *et al* (69) revealed that HMGB1 derived from NETs potentiates the degree of malignancy of cancer cells. Inhibition of HMGB1 by thrombomodulin inhibited NETs, hence impeding PC metastasis to the liver. However, the study of NETs is mostly performed using *in vitro* or murine models. Therefore, further investigations are required to determine whether these results are translatable to humans.

PCAT-1. Long noncoding RNAs (lncRNAs) are vital to tumor progression. Multiple lncRNAs have various pro-oncogenic functions in PC. For instance, HOTAIR, MALAT-1, ENST00000480739 and AFAP1-AS1 regulate cell invasion (70-73). The latter three lncRNAs are also promoters of cancer cell migration (74).

Using reverse transcription-quantitative PCR analysis, it was determined that upregulation of PCAT-1 inhibited the mRNA and protein expression of RBM5. In other words, knocking down PCAT-1 suppresses tumor cell migration and invasion (75). However, studies on PCAT-1 are currently scarce. Further investigation is required to fully explain the molecular mechanisms that drive tumor cell dissemination during cancer progression.

CA19-9. CA19-9 is a controversial biomarker. It lacks specificity in detecting PC. False-positive CA19-9 may be observed in obstructive jaundice even if successfully drained (76). Serum of patients that have biliary infection, inflammation or obstruction may test positive for CA19-9 (77,78). It was indicated to be associated with lymph node metastasis and unfavorable survival outcome in patients with colon cancer (79).

In the American Association of Clinical Oncology guidelines, CA19-9 is not recommended as a substitute for imaging for post-operative evaluation (11). CA19-9 is not a robust screening tool for PC and previous studies reported a low positive predictive value ranging from 0.5 to 0.9% (80,81). However, it may be utilized for screening at-risk individuals (82). New international guidelines for managing populations at high risk for developing familial PC recommend that CA19-9 testing should be performed when suspected, regardless of its uncertain diagnostic value (83).

In fact, screening of at-risk individuals has gained interest from researchers recently. Extensive studies have identified risk factors for PC. A meta-analysis concluded that diabetes mellitus is both an early manifestation and consequence of PC with a summary relative risk of 1.94 (95% CI, 1.66-2.27) (84).

Long-standing pancreatitis is proclaimed to be a strong risk factor (85). Though rarely observed, PC may result from mucinous pancreatic cysts (IPMNs) and mucinous cystic neoplasms (86).

The diagnostic value of CA19-9 for PC has been argued to be satisfactory. According to a previous evaluation, multiple tumor markers did not perform better than the single use of CA19-9 (87). However, this requires further confirmation.

CA19-9 does provide useful hints regarding prognosis (88,89). Certain studies concluded that CA19-9 was associated with poor survival outcome after pancreatic resection, with a cut-off value of 1,000 U/ml pre-operatively and 180 U/ml post-operatively (90,91). It was proposed that CA 19-9 may be utilized to predict surgical recurrence. The use of CA19-9 has been suggested as an effective prognostic marker in conjunction with S100A4 (92).

Despite all the controversies, the present review favors the utilization of CA19-9 in the screening, diagnosis, prognosis and surveillance of PC. While there is currently no consensus regarding the cut-off value of CA19-9 during these steps, nor any clear understanding of the relationship between CA19-9 and PC, it is likely to be beneficial to monitor CA19-9 together with other approaches.

FBXL7. The SCF E3 ubiquitin ligase family controls abundant protein degradation through the proteasome system and is pivotal in tumorigenesis and progression. However, the knowledge regarding their role in PC metastasis remains limited. Low FBXL7 mRNA and protein levels were observed in PC metastasis. Defects in the FBXL7-mediated degradation of c-SRC increase cell migration and invasion and the expression of EMT markers (93). Insignificant FBXL7 expression predicts poor survival. Previous work unveiled the anti-metastatic role of ubiquitin ligase subunit FBXL7 in pancreatic carcinoma using decitabine (a Food and Drug Association-approved DNA-methylase inhibitor to reduce metastasis) (94). FBXL7 promotes cancer cell invasion and metastasis through regulation of EMT, while EMT may be mediated by c-SRC. In numerous types of solid tumor, c-SRC expression levels are rather high and correlated with metastasis. FBXL7 was observed to coordinate c-SRC degradation and to further suppress the reduced EMT and tumor cell migration (93). Collectively, FBXL7 may be a candidate target for PC therapy.

PTX3. PTX3 belongs to the pentachlorobenzene toxin family and is synthesized in numerous cell types, such as endothelial cells, macrophages and monocytes. It has been reported that serum PTX3 is an important and specific biomarker for early infection (95). It helps with the diagnosis of PDAC and distinguish it from non-cancerous conditions such as intraductal papillary mucinous tumors or chronic pancreatitis (CP). PTX3 levels in blood samples from patients with PDAC, healthy volunteers and subjects with other non-cancerous diseases of the pancreas were measured by ELISA and patients with PDAC had significantly higher serum levels of PTX3 than patients with intraductal papillary myxoma or CP, and the sensitivity and specificity of PTX3 in detecting PDAC were better than those of serum CA19-9 and carcinoembryonic antigen. Goulart *et al* (96) advocated that PTX3 is a putative stromal-derived biomarker for PDAC, which warrants further testing in larger, prospective, multi-center cohorts and within clinical trials targeting stroma.

Tumor stroma. A dense stroma that blocks therapeutic agents is a typical hallmark of PC and this subsequently facilitates chemoresistance. Stroma depletion is an option to enhance therapeutic effects, which, in turn, hinders the stroma's role in tumor metastasis. Thus, it has been proposed to reshape tumor stroma to alter the communication between cancer cells and stromal compartments, eventually improving survival outcomes. Stromal-based therapies heavily rely on multiple elements of stroma, such as the extracellular matrix (ECM), immune cells, carcinoma-associated fibroblasts, blood and the lymphatic vasculature. It was argued that effects of ECM remodeling are not as promising as expected due to the heterogeneity of the tumor microenvironment (97). However, ECM alterations induce changes in the intra-tumor vasculature (98). In other words, such intricate interaction makes manipulation even better. Changes in either of them may affect stromal performance during cancerous progression and alter the outcome of malignancy. Theoretically, stroma depletion is a promising potential means of PC treatment. However, several clinical studies indicated that the combination of stromal depletion and chemotherapy was not beneficial (99-103). Of note, several studies using mouse models of PC exhibited undesired adverse effects, including cachexia, weight loss, hypoxia, increased immunosuppression and vascular density, loss of vascular integrity, an enhanced cancer stem cell-like phenotype and acidosis (103-106). To conclude, the stromal alteration strategy enhances the efficacy of therapeutic agent delivery but prior to its implementation, suppression of its side effects must be achieved first.

Non-coding RNAs. Non-coding RNAs are RNA molecules that are transcribed from genomes that do not code for proteins. They may be divided into two categories. In the first category, the role of the non-coding RNAs is to ensure that the basic biological functions are being performed and they are called constitutive noncoding RNAs. The other category is that of the macro-control noncoding RNAs (regulatory non-coding RNAs). Non-coding RNA participates in processes of various cellular functions, such as EMT, cell cycle control, apoptosis and autophagy (107).

miRNAs as a class of small non-coding RNAs regulate gene expression at the post-transcriptional level by binding to the 3'untranslated region of their target mRNAs. Altered expression of miRNAs has been indicated to be involved in the regulation of crucial pathological processes in tumorigenesis, progression and metastasis of PC (Table I). As a potential non-invasive biomarker for numerous cancer types, miRNA may be used as a diagnostic and prognostic marker for PC (108,109). Khan *et al* (110) demonstrated a significant upregulation of miR-215-5p, miR-122-5p and miR-192-5p, while the levels of miR-30b-5p and miR-320b were significantly lower in serum samples from patients with PDAC as compared to those from subjects with CP and healthy controls (HC). Receiver operating characteristic analysis indicated that these 5 miRNAs are able to distinguish PDAC from both CP and HC. Hence, this panel may serve as a non-invasive biomarker for the early detection of PDAC.

miRNAs may also be used as prognostic biomarkers. It was reported that high expression of miR-212 and miR-675 and low expression of miR-148a, miR-187 and let-7g in

Table I. Biomarkers for metastatic PC.

Noncoding RNAs	Biological function	(Refs.)
miR-21	Interferes with the expression of key self-renewal regulators (such as Oct4, Nanog and Sox2), degrades maternally inherited mRNA, initiates or promotes EMT, increases proliferation, invasion and chemoresistance	(114)
miR-24	Promotes cell proliferation and blood vessel formation, inhibits PANC1 cell apoptosis	(115)
miR-155	Promotes cell proliferation and tumorigenesis	(116,117)
miR-221/222	Promotes cell proliferation	(116)
miR-210	Inhibits tumor growth	(118)
miR-155	Promotes cell proliferation, inhibits apoptosis	(119)
miR-200b	Inhibits EMT and cancer cell migration	(120)
miR-196a-2/196	Promotes cell proliferation and inhibits cell apoptosis	(121)
miR-27a	Regulates tumor growth, colonization and migration	(122)
miR-506	Inhibits tumorigenesis	(123)
miR-301a	Promotes the proliferation of PC cells and inhibits their protein expression *in vitro* and *in vivo*	(124)
miR-98-5p	Promotes the proliferation and metastasis of PC cells	(125)
miR-377	Inhibits the growth and migration of pancreatic tumors and induces apoptosis	(126)
miR-135a	Inhibits cell proliferation	(8)
miR-203-3p	Inhibits the expression of fibroblast growth factor 2; regulates the proliferation, invasion and migration of PC cells	(127)
miR-125a-3p	Suppresses EMT	(128)

PC, pancreatic cancer; miR, microRNA; EMT, epithelial-mesenchymal transition.

non-microanatomical carcinoma tissues from patients undergoing PC surgery was able to predict OS, and high expression of miR-155, miR-155, miR-203, miR-210 and miR-222 in pancreatic tumors was associated with a low survival rate (111,112). Furthermore, low expression of miR-7 was reported to be associated with poor prognosis and to accelerate tumor progression in PC (113).

5. Conclusion

The goal of early detection of metastatic PC is laudable. Obstacles are the relatively low prevalence of PC (and even smaller subpopulations), resulting in a less feasible screening protocol for the general population. Biomarkers for early detection remain to be validated. Unveiling the roles of signaling pathways in PC may be insufficient for the timely diagnosis of PC. Novel combinations with imaging modalities with state-of-the-art robust algorithms may clearly determine the anatomical structure and pathological changes for this disease.

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Availability of data and materials

Data sharing is not applicable.

Authors' contributions

XC and FX drafted the manuscript, FL drew the figure, and QX and XW collected the references and extracted the necessary data. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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