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Advances in Biomarker Research for Pancreatic Cancer

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Abstract

Pancreatic cancer (PC) is a leading cause of cancer related deaths in United States. The lack of early symptoms results in late-stage detection and a high mortality rate. Currently, the only potentially curative approach for PC is surgical resection, which is often unsuccessful because the invasive and metastatic nature of the tumor masses makes their complete removal difficult. Consequently, patients suffer relapses from remaining cancer stem cells or drug resistance that eventually lead to death. To improve the survival rate, the early detection of PC is critical.

Current biomarker research in PC indicates that a serum carbohydrate antigen, CA 19-9, is the only available biomarker with approximately 90% specificity to PC. However, the efficacy of CA 19-9 for assessing prognosis and monitoring patients with PC remains contentious. Thus, advances in technology and the detection of new biomarkers with high specificity to PC are needed to reduce the mortality rate of pancreatic cancer.

Keywords

Pancreatic cancer; biomarker; tumor progression; risk factor; CA 19-9

INTRODUCTION

With a mortality rate of nearly 100 %, pancreatic cancer (PC) is one of the most lethal malignancies [1] and it is the fourth most prominent cause for cancer-related deaths in both men and women in the United States [2–3]. Even patients treated with current state-of-the-art therapies have an overall 5-year survival rate of only 3% [4]. The main reason is that approximately 80% of newly diagnosed patients are already in the metastatic stage of the disease, for which no curative therapy is currently available [5–7].

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Most patients with PC have no distinct clinical manifestations in their early stages of the disease. Commonly described symptoms include pain in the upper abdominal region, significant weight loss, heartburn, altered bowel habits, and obstructive jaundice [8]. Obstructive jaundice is the only symptom with some specificity for PC [9–12]. The diagnosis of PC relies on biopsy and computed tomography (CT) and/or magnetic resonance imaging (MRI) [13–14]. The diagnosis could be improved by using endoscopic ultrasound (EUS) and positron emission tomography (PET) techniques [15–17]. Advanced technologies such as magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP) facilitate better detection of tumors at an early stage by allowing a comprehensive and introspective analysis of the morphological changes of the gland [18–20]. Other techniques like fine-needle aspiration (FNA) are useful for pinpointing and retrieving biopsied specimens [21–22]. However, these techniques are not only costly but also require access to highly advanced, specialized facilities (Table **1**), and therefore may not be readily available for detecting the disease at an early stage.

At present, no curative therapies are available for patients with PC; the mainstay of treatment with the potential for cure is the complete, eradicative surgical resection of the primary carcinoma. Due to the lack of specific early-stage clinical symptoms and the highly deleterious, aggressive nature of the evolving disease, patients are usually diagnosed at the unresectable stages [23–24]. Consequently, the average survival of the resected patients is approximately 12 to 20 months with a high probability of relapse [25–26]. Use of gemcitabine (Gemzar®) or the combinations of gemcitabine with other cancer drugs, such as oxalipatin, cisplatin or erlotinib has demonstrated efficacy in the treatment of PC [27–30]. The role of radiotherapy in PC treatment remains controversial [31], although radiochemotherapy is increasingly accepted as a treatment modality for unresectable, nonmetastatic PC [32–33]. Despite these advances, PC remains one of the most deadly malignancies.

TUMOR ORIGIN AND PROGRESSION

Although the exocrine pancreas consists of a very small ductal system, 90% of pancreatic neoplasms (of which 80% are invasive adenocarcinomas) are ductal in origin, and the remaining 10% are islet-cell and cystic tumors [1]. The etiology of PC remains unclear, but several risk factors have been identified (Table **2**). In general, individuals diagnosed with diabetes or with a history of pancreatitis or chronic pancreatitis are at a risk for developing PC [1, 34–35]. A history of smoking and/or alcoholism also increases the risk of PC [36–38]. Recently, Peutz-Jeghers syndrome, single nucleotide polymorphisms (SNPs), hereditary pancreatitis, and a 5–10% family history of PC have also been added as PC risk factors [16, 35, 39].

More than 90% of PC patients acquire the disease sporadically; it is unclear how the normal tissue is transformed to PC. Fig. (**1**) illustrates a Genetic Progression Model based on the oncogenic markers in tumor tissues [1, 40–41]. In the model, normal pancreatic cells are transformed to pre-malignant lesions termed pancreatic intraepithelial neoplasia (PanINs) in concomitance with a multitude of genetic changes and morphological dysplasia, named PanIN-1a, PanIN-1b, PanIN-2, and PanIN-3 that collectively may be regarded as molecular and structural antecedents to the eventual establishment of invasive adenocarcinoma [10,11]. Some of the most frequently detected genetic changes in the initial stages of PC include the activation of mutated K-ras [42–44], the hypermethylation of preproenkephalin (ppENK) [45–46], the over-expression of EGF-family ligands and receptors such as ERBB2 and EGFR [47–49], the robust increase in the level of carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) (CEACAM1) [50], and the hyperphosphorylation of Retinoblastoma protein (Rb) [51]. Most notably, some researchers hypothesize that the

activation of K-ras mutation signaling, leading to the overexpression of EGF-family ligands and receptors such as ERBB2 and EGFR, plays a pivotal role in the initiation of PC [47–49]. During disease progression, the loss of tumor suppressor genes (e.g. p16, p53, SMAD4/DPC4, BRCA2) has been identified at later stages of PanIN [52–58]. The over-expression of a negative regulator of p53 called *MDM2* occurs in coordination with the malignant proliferation of PC [59–60]. These molecular changes and events as a whole promulgate the hyperphosphorylation of Rb protein, which promotes cell cycle phase transition and leads to uncontrolled cell division [51]. Along with tumor progression, the expression of *CXCR4*, a chemokine receptor, and miR-155 also increases in PC especially in the PanIN-3 lesions [41, 61–62]. Further, the level of macrophage stimulating 1 receptor (*MST1R*), also termed RON, is increasingly elevated in premalignant PanIN stages [63]. Additionally progressive methylation in the promoter regions of *LHX1*, a transcriptional regulator and secreted apoptosis related protein (*SARP2*), has also been reported in PanIN lesions [64–66]. The aggressive stage of the tumor is also characterized by the appearance of abnormally shortened telomere [67] and the abnormal fusion of chromosome ends, concurrent with neoplastic progression of pancreatic cells [68].

ADVANCES IN PC BIOMARKER RESEARCH

Improving the PC mortality rate necessitates the discovery of new tools for the early detection, diagnosis, and monitoring of therapeutic efficacy. To provide a context, we provide an overview of technological developments relevant to biomarker research and biomarker identification in the early stages of PC.

TECHNOLOGIES FOR BIOMARKER DISCOVERY AND IDENTIFICATION

Important technological innovations have been made in the past several decades to facilitate biomarker identification. Microarray and quantitative RT-PCR or PCR are frequently used to analyze the variations of RNA/microRNA or DNA in the tumor tissues and other types of specimens from patients and healthy individuals [69–71]. Methylation-specific PCR (MSP) and bisulfite-sequencing PCR (BSP) [72] have been used to analyze DNA from different samples [73]. Gene expression profiling and tissue microarray (TMA) have been used to identify novel cell-surface targets [36]. New equipment and techniques have been developed to systematically search for protein markers in specimens, including differential in-gel electrophoresis (DIGE), tandem mass spectrometry (MS/MS), electron transfer dissociation mass spectroscopy [74], surface-enhanced laser desorption/ionization (SELDI) [75–76], reverse-phase protein array [74], enzyme linked immuno sorbent assay (ELISA), matrix assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF-MS), and quantitative proteomics [77–78]. Advances in these technologies have provided significant leads in search of potential biomarkers for PC (Table **3**).

SOURCE OF BIOSPECIMENS IN BIOMARKER ANALYSIS

Sample source is important in the search for biomarkers to detect and diagnose early PC. Pancreatic biopsy samples are reliable sources for diagnosis, and pancreatic fluid (known as juice) from the pancreatic duct has recently been identified as an alternative source for biomarker discovery [72, 79] because the juice contains PC-specific markers (DNA/RNA/proteins and cancer cells) that are shed into the ductal lumen [79] (Table **4**). Furthermore, plasma/serum DNA/RNA/protein, disseminated tumor cells (DTC) and circulating tumor cells (CTC) in the blood are also frequently used for biomarker detection. In addition, saliva has also been exploited as a source of PC biomarkers and genes have been detected with potential for classification of resectable PC [80]. A recent study has suggested that detection of genetic markers such as K-ras and p53 in stool samples might enable the detection of

early PC [81]. Potential biomarkers for PC using various source materials and discovered thus far are summarized in Table **4**.

BIOMARKERS

With the aid of new technologies and using a variety of sample sources, a wide array of potential biomarkers for PC has been identified (Table **5**).

GENETIC BIOMARKERS

Based on the tumor progression model, multiple genetic alterations are involved in a step-wise manner in the development of PC and in most cases, genetic changes in the four genes – *K-ras*, *CDKN2A/p16, TP53,* and *SMAD4/DPC4* were detected in the PC patients [5, 22, 82–86]. Among these genetic changes, the *K-ras* mutation was most frequently detected with 45%–100% frequency in the tumor tissue and a 77% frequency in the serum of patients with PC or pancreatic adenocarcinoma (PAC) [22, 43, 87–88]. 50–75% patients with PAC have mutations in *TP53* [43, 89]. In addition to *K-ras* and *TP53* mutations, the loss of p16 and DPC4 was also frequently detected in tumor tissue of PC patients and correlated with its clinical relevance [84, 90–93]. The *K-ras* mutation was detected in the premalignant stage, which suggested that the oncogenic gain of mutation in *K-ras* could be a potential marker used for the early detection of cancer [94–96]. Other genetic alterations described in PC occurring at a much lower frequency include *BRCA2, TGFBR1, BAX, RB1, STK11, hMLH1, hCDC4, MKK4*, *FancC,* and *AKT2* [94, 97–99]. Another biomarker *Plectin-1* that was highly expressed in the PanIN-3 tissues may be used to discriminate the primary from metastatic PAC [100]. Studies using a functional genomics approach identified seven genes from plasma – *TNC, TFP1, TGFBI, SEL-IL, LICAM, WWTR1,* and *CDC4BPA* – as belonging to a category that was differentially regulated in PC versus normal samples. Studies also suggested that tissue factor pathway inhibitor (TFPI) may have the potential to be a candidate plasma PC biomarker [101–102]. It is worth noting that although genetic alterations in oncogenes - *K-ras*, *TP53, p16* can be detected in PC [103] and may have diagnostic value in PC, they are not specific to PC and hence not suitable for the early detection of this deadly malignant disease.

EPIGENETIC BIOMARKERS

Epigenetic factors such as aberrations in DNA methylation, histone post-translational modification, and chromatin remodeling could also be considered as tumorigenic biomarkers [104]. Global methylation profiling on 150 CpG sites across 807 genes revealed that 289 CpG sites were differentially methylated between normal and PC, raising the possibility that they have diagnostic and therapeutic values and implications [105]. Genome-wide profiling of methylated promoters in 57 PC and 34 normal pancreatic samples identified excessive methylation on the promoters of several genes, including *MDF1*, hsa-miR-9-1, *ZNF415, CNTAP2* and *ELOVL4* in cancerous tissues [106]. Further, frequent epigenetic changes have also been demonstrated in PC in *RASSF1A, cyclin D2, ppENK, TFPI2, CDH13* and secreted apoptosis related protein 2 *(SARP2)* [45, 64, 99, 107–115], suggesting that epigenetic profiling of selected genes in tissue specimens could be used for PC diagnosis and prognosis. A similar approach applied to the analysis of pancreatic juice showed that over 1% in the methylation status of *cyclin D2, ppENK,* tissue factor pathway inhibitor 2*(TFPI2),* neuronal pentraxin-2*(NPTX2)* and forkhead box protein *(E1FOXE1)* could predict PC with 82% sensitivity and 100% specificity [99]. The hypermethylation of *NPTX2* and *SARP2* in PC patients was recently confirmed [115–116]; similarly, methylation profiling analysis of circulating plasma DNA in PC patients detected *cyclin D2* and a few other markers in PC patients [117]. Other epigenetic modifications potentially useful as

biomarkers for poor survival in resected PAC patients include low cellular levels of histone H3 lysine 4 dimethylation (H3K4me2), histone H3 lysine 9 dimethylation (H3K9me2), and histone H3 lysine 18 acetylation (H3K18ac) [118]. Epigenetic silencing of *SIP1* and hypermethylation and over-expression of miR-200a/200b in association with elevated circulating miRs have been reported in PC [119].

MIRNA BIOMARKERS

miRNAs are short non-coding RNAs of approximately 22 nucleotides that have been shown to regulate the expression of both oncogenes or tumor suppressor genes, as part of the posttranscriptional gene silencing (PTGS) mechanism [120–124]. Recently, it was found that the levels of certain tissue-specific miR-NAs, such as miR-216 and -217, were markedly down-regulated in tumor tissues compared to matched normal controls [125]. Conversely, the expression of miR-196 and -196a was significantly elevated in tumor tissues derived from PAC patients compared to normal pancreatic specimens [125]. Moreover, miR-196a was detected in the serum of PAC, and thus may have predictive potential regarding the survival of PAC patients [126]. Other miRNA biomarker candidates include elevation in circulating miR-210 for PC [127], miR-21 as an integral link to the precursor lesions of PAC [128–130], and upregulation in an eight-miRNA panel (miR196a, miR-190, miR-186, miR-221, miR-222, miR-200b, miR-15b, and miR-95) based on an RT-PCR analysis involving 5 PC tissues and 3 PC cell lines spanning a total of 95 microRNAs [131].

OTHER BIOMARKERS

Beyond targeting genetic, epigenetic and miRNA markers in PC using primarily tumor specimens, with some less well defined and specific changes detected in the serum and other body fluids, several other PC biomarkers have been identified and are listed in Tables **5** & **6**. Among these, perhaps the one considered to have the most definitive diagnostic, prognostic, and predictive value is carbohydrate antigen 19-9 (CA 19-9), which shows an 81% sensitivity and 90% specificity [132–137].

CA 19-9

Although first described as a tumor-associated carbohydrate antigen in 1979 by Koprowski *et al.*, [138–139], CA 19-9 is now known to be a sialylated Le^a blood group antigen [140]. The nomenclature of CA 19-9 was compiled from the mucinous glycoprotein complex (such as H-CanAg, L-CanAg, and MUC1) [133, 141] based on recognition of sialylated lacto-N-fucopentaose II epitope by the moloclonal antibody (1116-NS-19-9). The levels of CA 19-9 in the serum varied according to the patient's secretor status and Lewis genotype [139–140, 142–143]. Individuals who are Lewis blood type negative (Le^{a-b-}) do not express the CA 19-9; hence, for those diagnosed with PC in this group (about 5% of the PC population), elevation in CA19-9 is not observed because of deficiency of a fucosyltransferase essential for the biosynthesis of CA19-9 and the Lewis antigen [144].

CA 19-9 was initially detected in colorectal cancer cell line SW1116 and colorectal tissues [139] but its occurrence was subsequently extended to other organs such as pancreas, stomach, and biliary epithelium [145]. Based on reports showing that the sialyl $Lewis^a$ structure (NeuNAcα2-3Ga1β1-3Glc [4-Fucα1] NAcβl-3Galβl-4Glc) acts as a ligand for E-selectin [140, 146–148], Kannagi proposed that three factors should be taken into consideration when predicting the risk of hematogenous metastasis in cancer patients: the degree of sialyl Lewis a/x expression on cancer cells, the degree of E-selectin expression on vessel walls, and the number of CTC in the bloodstream [147]. The concentration of CA 19-9 can be quantitatively determined by CA 19-9 ELISA [101]. Elevated levels (>37 U/ml) of CA 19-9 have been associated with gastrointestinal carcinomas, particularly in PAC [144,

149–151], and may be considered as a well characterized biomarker for PC (reviewed by Duffy *et al.*) [152], and it is possible to predict the operability of pancreatic tumor in patients. For instance, elevated levels of CA 19-9 (1000 U/ml) are correlated with patients showing a tumor size >5cm in diameter [153]; only 5% of those patients have resectable tumor [134, 153–154]. Other studies have shown that serum CA 19-9 concentrations greater than 300 U/ml are indicative of unresectable PC tumors [155–156].

An increase in CA 19-9 levels may also be used to predict cancer progression while its decrease is suggestive of favorable response to neodjuvant irradiation and chemotherapy [157]. Steinberg compared CA 19-9 levels in 24 studies and found that CA 19-9 showed 81% sensitivity and 90% specificity for PC with a 37 U/ml cut-off [134]. Similar conclusions were reached by Goonnetilleke and Siriwardena [158]. Taken together, therefore, the immuno quantitation of CA 19-9 antigen is regarded to be the gold standard for PC detection [152, 159]. Reservation in using CA 19-9 alone for PC diagnosis has been raised by European Group of Tumor Markers (EGTM) [152, 160], and by the National academy of Clinical Biochemistry (NACB) [152, 161]. Some noted caveats include: elevated CA 19-9 can be similarly detected in non-malignant patients diagnosed with acute and chronic pancreatitis, obstructive jaundice, and liver cirrhosis [134, 152, 162], and in gastrointestinal carcinomas [134, 158, 162–163]; furthermore, false positive results may result based on detection of a low molecular weight antigen, non-CA 19-9 [164]. Thus, the European Group of Tumor Markers (EGTM) [152, 160] the National academy of Clinical Biochemistry (NACB) recommend that CA 19-9 should not be the only indicator used for diagnosing PC.

Other potential PC biomarkers include (1) elevation in MMP-7 in plasma and pancreatic juice to discriminate between benign disease and carcinoma when combined with CA 19-9 [165], (2) a 2–10 fold increase in AGR2 in pancreatic juice, for the early detection of PC [166], (3) changes in S100A6 in pancreatic juice, for early detection of the disease [167], (4) elevated telomerase activity in pancreatic juice [168], (5) serine proteinase-2 (PRSS2) pre-protein and pancreatic lipase related protein-1 (PLRP1) in pancreatic juice of PC patients [72], and perineural invasion in PC [169]. Recently, a few studies have investigated whether use of biomarker panels may enhance the specificity and improve the diagnosis of PC [170–171]. The results showed that grouped biomarkers are comprised of CA 19-9, MCSF, CEA, SAA, Haptoglobin, TSGF, CA 242, and HSP27 only showed comparable sensitivity and specificity to single serum markers [170]; in contrast, combined assessment of CA 19-9, ICAM-1, and OPG gave 78/94% sensitivity/specificity and represented a significant improvement over individual CA 19-9 measurements for differentiating PC patients from healthy controls and subjects diagnosed with benign disease [171].

CANCER STEM CELLS AND CTC

The prevention of tumor relapse in PC has not been successful [172–173]. The underlying mechanisms are multi-faceted and may be attributed to drug resistance and insensitivity to repeated treatments, as well as the presence of pancreatic cancer stem cells (CSC) [174] which provide gain-of-function via their ability to undergo self-renewal, differentiation [175], and metastasis [172]. Accordingly, several recent studies have confirmed the presence of CTC in the blood stream and utilized CTC in the tumor tissue as a novel approach to develop biomarkers for PC. Experiments have shown that the presence of CTC might prove useful for prognosis in PC patients [176–178]. Although preliminary results from studies of CSC and CTC are encouraging, the technologies required for the capture of CSC or CTC are still at an early stage for routine application in PC diagnosis.

Likewise, mixed lymphocytes have shown promise for predicting patient's outcome in PC. For instance, an overall survival of 2.4 months was predicted in PC patients based on an elevated neutrophil to lymphocyte ratio [179]; the elevated levels of micronuclei in peripheral lymphocytes may have an association with PC risk [180]. Experimentally, identity of mixed lymphocytes may be validated by the RT-PCR amplification of CK-19 in blood [181]. However, the specificity of these measurements may be confounded by innumerable factors that affect the lymphocyte populations in the blood stream.

FUTURE PERSPECTIVES

In conclusion, the establishment of early diagnostic biomarkers is critical to increase survival of PC patients. So far, no single marker has been identified with the specificity and reliability needed for screening PC during the early stages of the disease, even though a wide array of serum and tissue-based candidate markers have been identified. Biomarkers in body fluids have considerable potential for clinical diagnosis and prognosis; however, their application is limited by their low concentration compared to what might be available in the tumor tissue. The possibility that CA 19-9 may prove beneficial in diagnosing PC is supported by its 90% specificity to PC, but its reliability is still open to debate. Furthermore, more basic studies using cell lines, animal model experiment systems, and patient samples will be required to better understand the PC biology as a necessary requisite to identify more specific biomarkers for PC. The development of new methods to detect potential biomarkers with higher specificity and accuracy even for low concentrations in the body fluids would further advance biomarker research.

Promising new area for PC biomarker research and application lies in an in-depth analysis of CTCs and CSCs. New technologies and approaches must be developed for isolating these cells, keeping them in culture without changing their genotype or phenotype, and understanding the roles which they might play in the genetic evolution of PC, will go a long way in facilitating PC diagnosis and developing novel management and treatment strategies.

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ABBREVIATIONS

PC	Pancreatic cancer
CT	Computed tomography
MRI	Magnetic resonance imaging
EUS	Endoscopic ultrasound
PET	Positron emission tomography
MRCP	Magnetic resonance cholangiopancreatography
ERCP	Endoscopic retrograde cholangiopancreatography
FNA	Fine-needle aspiration

PanIN	Pancreatic intraepithelial neoplasia
SNP	Single nucleotide polymorphism
MSP	Methylation specific PCR
BSP	Bisulfite-sequencing PCR
TMA	Tissue microarray
DIGE	Differential in-gel electrophoresis
MS	Mass spectrometry
SELDI	Surface enhanced laser desortion/ionization
ELISA	Enzyme linked immune sorbent assay
MALDI	Matrix assisted laser desorption/ionization
DTC	Disseminated tumor cells
CTC	Circulating tumor cells
PAC	Pancreatic adenocarcinoma
PTGS	Post transcriptional gene silencing
CA 19-9	Carbohydrate antigen 19-9
EGTM	European group of tumor markers
NACB	National academy of clinical biochemistry
CSC	Cancer stem cells
2DE	2 dimensional electrophoresis
MudPIT	Multi-dimensional protein identification technology
ICAT	Isotope coded affinity tag
iTRAQ	Isobaric tags for relative and absolute quantification

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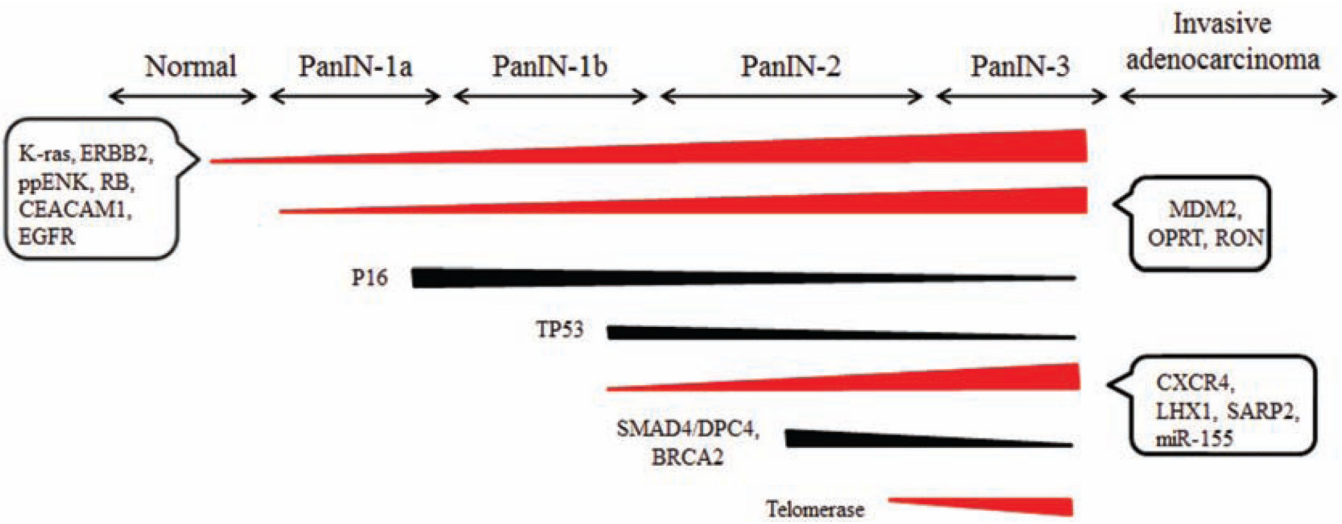

Fig. (1).
Genetic progression model for PC [1, 40–41].

Table 1

Advances in Diagnostic Tools for PC [14–16, 19–20, 182–187]

Diagnostic Tool	Applications and Advantages	Limitations
Transabdominal ultrasound	Visualization of bile duct and liver	Poor visualization of pancreas
Computed tomography (CT)	Diagnosis and staging	Nephrotoxicity (Iodine-contrast agent); potential side effects from exposure to radiation
Magnetic resonance imaging (MRI)	Superior imaging of pancreas and bile duct Iodine-free contrast agent No radiation	Expensive Less availability
Positron emission tomography (PET)	Metastatic disease assessment, Monitoring recurrence and response to adjuvant therapy	Expensive Less availability
Endoscopic ultrasound (EUS)	Imaging of pancreatic masses and lymph nodes	Expensive Less availability
EUS-FNA (Fine needle)	Specific detection of PC Safe and less invasive technology Imaging of lesions from 3–4 mm upwards Puncturing of lymph nodes greater than 5 mm	Access only to posterior mediastinum
Magnetic resonance cholangiopancreatography (MRCP)	Imaging of fluid in the pancreatic ducts in a non-invasive manner	Lower resolution No biopsy Expensive
Endoscopic retrograde cholangiopancreatography (ERCP)	Imaging strictures	Complications like bleeding and infection
Cholangiopancreatoscopy	Site-directed biopsies of strictures	Fragile equipment

Table 2

High Risk Factors for PC

Risk factors	References
Obesity (Body Mass Index) ≥ 30	[188–190]
Having symptoms indicative of PC for ≥ 3 years	[1]
Smokers & smokers specifically with polymorphism at $Arg^{188}His$	[34–35, 38, 191–192]
Obstructive jaundice	[1, 159]
Onset of type II diabetes for ≥ 3 years	[1, 193–194]
Chronic pancreatitis & pancreatitis history	[195–196]
Peutz-Jeghers syndrome	[197]
Hypermethylation of p14 and p16 + K-ras mutation	[198]
ABO blood group	[199–201]

Table 3

Current Technologies for Biomarker Research [202–212]

Technology		Applications and Advantages	Limitations
Microarray		Chip-based concurrent analysis of thousands of genes	Frequent identification of false positives
RT-PCR/PCR/ MSP		Multiple target analysis in a single, multiplexed reaction	Sensitive, can generate false positive results
PROTEOMICS	Two-dimensional gel electrophoresis (2DE)	Separation of proteins by isoelectric point (IP) and molecular weight (MW)	Permits detection of very basic or acidic proteins only
	Differential in-gel electrophoresis (DIGE)	Co-separation of multiple samples and visualization on one 2-D gel	Changes in labeling can markedly alter position of migration of specific proteins
	Multi-dimensional protein identification technology (MudPIT)	Non-gel approach for the identification of proteins from complex mixtures	Inability to directly measure the relative abundance of proteins
	Isotope coded affinity tag (ICAT)	Measurement of relative abundance of proteins labeled at cysteine residues	Suitable only for proteins with labeling-accessible cysteines
	Isobaric tags for relative and absolute quantification (iTRAQ)	Identification and quantification of proteins by labeling both whole and subcellular proteomes	Unlikely to reveal basic information on proteins of interest
	Matrix-assisted laser desorption/ionization (MALDI)	Identification of proteins isolated through gel electrophoresis	Quantitation difficult
	Surface-enhanced laser desorption/ionization (SELDI)	Analysis of protein mixtures by mass spectroscopy ionization method	Low reproducibility Low resolution

Table 4

Various Tissue Types Used for PC Biomarker Research

Sample Source	Detection Method	Markers	References
Tissue	Immunohistochemistry, PCR-based techniques, and Microarray	K-ras, TP53, SMAD4, p16, Plectin-1, Caveolin-1, hENT1, miR-216, miR-217, miR-196, miR-196a, miR-186, miR-222, miR-200b, miR-15b, miR-95, FosB, KLF6, ATP4A, NFKBIZ, GSG1, SIGLEC11	[22, 43, 84–85, 100, 125, 131, 213–220]
Pancreatic juice	Quantitative proteomics	AGR2, S100A6, MMP-9, DJ-1, A1BG, MMP-7, miRNA-21, miRNA-155, Twist, PRSS2, PLRP1, hTERT	[72, 166, 221–226]
Serum	Quantitative proteomics, ELISA,	CA 19-9, CA 125, CEACAM1, MMP-7, REG4, HSP-27, IGF, IGFR, cyclin I, GDI2, PF4, SAA, fibrinogen γ	[37, 50, 227–236]
Plasma	Functional genomics	TNC, TFP1, TGFBI, SEL-1L, LICAM, WWTR1, CDC4BPA, CK 18	[101, 237]
CTC	RT-PCR	C-MET, h-TERT, CK20, ELC, PlGF	[238–239]
Saliva	Human genome array	K-ras, MBD3L2, ACRV1, DPM1	[80]
Stool	PCR	K-ras, TP53	[81, 240]

Table 5

Most Studied Markers in PC

Biomarkers	Detection Method	References
Molecular biomarkers		
K-ras	PCR	[22, 43, 87, 241–243]
p16	PCR and immunohistochemistry	[22, 90, 213, 244–246]
P53	PCR and immunohistochemistry	[43, 87, 90, 242]
DPC4	PCR and immunohistochemistry	[93, 247]
Epigenetic biomarkers		
ppENK	Methylation specific PCR	[45, 99, 107, 241]
RASSF1A	Methylation specific PCR	[108–111]
Cyclin D2	Methylation specific PCR	[99, 112, 248]
MicroRNA biomarkers		
miR-155	Microarray and qRT-PCR	[41, 218, 224, 249–251]
miR-21	RT-PCR	[128, 130, 252]
miR-196a	Immunohistochemistry	[126, 131, 216, 249]
Other biomarkers		
CA 19-9	ELISA	[132, 253–256]
CEACAM1	ELISA	[50]
AGR2	RT-PCR, ELISA and Western blotting	[166, 257]
CEA	Immunohistochemistry	[236, 253, 258–260]
CA 125	Immunoradiometric assay and Immunohistochemistry	[260–261]
CA242	ELISA	[236, 253, 262–264]

Table 6

Most Promising Protein Markers for PC in the Serum

Markers	Screening	Diagno-sis/Prognosis	healthy Individual	Patient	Sensitivity	Specificity	References
CA 19-9	No	Yes	< 37 U/ml	> 37 U/ml	81%	90%	[1, 134, 158, 170, 265–266]
CEA	No	Yes	< 5 U/ml	> 50 U/ml	45%	75%	[158, 161, 236, 266]
CA 125	No	Yes	< 35 U/ml	> 35 U/ml	60%	80%	[260, 267]
CA 242	No	Yes	<25 U/ml	> 25 U/ml	60%	80%	[170, 236, 253, 264, 268–269]