Advances in pancreatic cancer biomarkers

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Abstract

Biomarkers play an essential role in the management of patients with invasive cancers. Pancreatic ductal adenocarcinoma (PDC) associated with poor prognosis due to advanced presentation and limited therapeutic options. This is further complicated by absence of validated screening and predictive biomarkers for early diagnosis and precision treatments respectively. There is emerging data on biomarkers in pancreatic cancer in past two decades. So far, the CA 19-9 remains the only approved biomarker for diagnosis and response assessment but limited by low sensitivity and specificity. In this article, we aim to review current and future biomarkers that has potential serve as critical tools for early diagnostic, predictive and prognostic indications in pancreatic cancer.

Introduction

Pancreatic ductal adenocarcinoma (PDC) is the most common subtype of pancreatic cancer, estimated around 85%.[1] Age-standardized incidence rate of PDC is 7.2 to 2.8 per 100,000 in developed region *versus* less developed[2] countries. Northern America considered having highest incidence rate globally while Africa has the lowest rate 7.4 and 2 per 100,000 respectively. Globally the mortality rate coincides with the incidence rate, emphasizing the poor prognosis. In United States, pancreatic cancer is the 4th leading cause of cancer-related mortality.[3] Recent epidemiology studies show that the incidence of new pancreatic cancer has been gradually increased over time[4] and within a decade, it is expected to rise to the second leading cause of cancer-related mortality behind lung cancer. In recent times, five years survival rate is minimally improved and reaches only 7% among all stages of pancreatic cancer.[5] The screening programs for PDC remains challenge compared with other tumors-lung, breast, colon and cervix. The barriers to develop screening test to detect pancreatic cancer include specificity of the chosen test and the relatively low incidence of the disease. This can lead to multiple false positive cases and further challenged by the cost and morbidity associated with invasive confirmatory testing. To overcome this in unselected patient population, a high performing screening test with sensitivity and specificity close to 100% is required. Current attempts to discover screening tests in PDC for early diagnosis have focused mainly on serum biomarkers. According to national cancer institute, the biomarker has been defined as *any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease*. The carbohydrate antigen (CA) 19-9, the only biomarker approved by US Food and Drug Administration (FDA) is not considered as a screening tool due to its low sensitivity and specificity.[6,7] It was reported previously that the median sensitivity for CA19-9 was 79% while median specificity was 82%.[8] Therefore, the screening efforts were directed on the high-risk groups with familial risk and chronic pancreatitis; however, these represent the minority of affected individuals. Applying screening strategies to patients with one or more of the risk factors could enhance the performance of a putative screening test. Biomarkers role is crucial in diagnostic and therapeutic approach in cancer treatment and are key assets in identification of a sub-group of population to target preventive interventions.[9] In the sporadic pancreatic cancer group, no biomarkers so far with high enough accuracy are currently available for use in screening and therefore an urgent unmet need for identification of right biomarker.[9] The aim of this article was to review the novel biological and molecular biomarkers with diagnostic, predictive and prognostic potential in PDC patients.

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Diagnostic markers

Most patients with early-stage PDC are asymptomatic, however commonly diagnosed at advanced stages, where the treatment options are limited and associated with worse clinical outcomes. The poor prognosis of PDC attributed to late diagnosis with advanced presentation, where curative therapeutic options are lacking. Identifying robust biomarkers for earlier detection could enable management of these cancers with curative intent and thus reducing the PDC mortality. To date, there is no biomarker approved for early diagnosis. This underscores the unmet need for development of early detection biomarkers.

Carbohydrate antigens and carcinoembryonic antigen

Serum carbohydrate antigen (CA) 19-9 is the most common and validated diagnostic tumor marker with sensitivity and specificity of 79-81% and 82-90% respectively; but have poor predictive value of 0.5-0.9% in asymptomatic patient.[10] CA 19-9 can be elevated in other medical conditions such as acute cholangitis, pancreatitis, obstructive jaundice and liver cirrhosis. Additionally, Lewis-negative blood type patient, which makes 5-10% Caucasian, do not produce CA 19-9 levels,[10] thus contributing to

false negativity. Currently, CA 19-9 is being applied in clinical practice for prediction of treatment response and prognostication. Few other carbohydrate antigens have also been studied extensively including CA-242, CA 50, CA 195, CA 72-4, CEA and CA-125, and found to be overall less sensitive than CA19-9.[11,12]

MicroRNAs

MicroRNA (miRNAs) belongs to a class of non-coding RNA that involve in expression of post-transcriptional regulatory mechanisms. Use of miRNAs expression profiling has gained importance as a biomarker for early detection of cancer.[13,14] In pancreatic cancer, miRNAs dysregulation has been profiled in pancreatic tissue, blood, stool and saliva.[15] Among several different miRNAs, miR-21, miR-155 and mi-R 196 have been demonstrated to be upregulated in PDC and can differentiate from pre-cancerous lesions as weli.[16-19] Since specimen acquisition from pancreatic juice and pancreatic tissue, requires invasive approach, non-invasive techniques such as fecal and urinary specimen has been studied for diagnostic purposes. Three miRNAs (miR-143, miR-223 and miR-30e) as assessed in urine samples were over expressed in stage I cancer as compared to healthy individuals. Additionally, miR-223 and miR-204 could distinguish early stage cancer from chronic pancreatitis. Furthermore, combination use of miR-143 and miR-30e achieved sensitivity of 83.3% and specificity of 96.2%.[20] Similarly, higher levels of miR-21 and miR-155 levels in PDC compared to normal controls was reported in stool specimen.[21]

Macrophage inhibitory cytokine 1

Macrophage inhibitory cytokine 1 (MIC-1) is an autocrine regulatory molecule, which distantly belong to transformer growth factor beta (TGF-β) superfamily. Serum MIC-1 levels may serve as a novel diagnostic biomarker for early detection of pancreatic cancers. A study by Koopman *et al.* demonstrated that serum MIC-1 outperform all serum markers including CA 19-9 levels in distinguishing resectable pancreatic cancer from healthy controls.[22] Recent studies including meta-analysis showed, serum MIC-1 levels were higher in pancreatic cancer patients as compared to controls.[23,24]

PAM 4

PAM4 is a murine monoclonal antibody (mAb) is reactive to Mucin 5 AC, a secretory mucin. The expression of PAM4 is highly restricted to early stages of neoplastic development in pancreas, including pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasm (IPMN).[25] PAM4 antibodies were found to be absent in normal pancreatic tissues and solely expressed in pancreatic cancers or those with early neoplastic changes. Gold et.al reported higher specificity of 85% for PAM4 alone in comparison CA 19-9 with 68%.[26] Interestingly, combined PAM4/CA19-9 assay reported to have improved sensitivity (84%) for early detection of PDC along-with improved specificity (82%). Additionally, PAM4 has been radiolabeled to enhance diagnostic accuracy[27] by radio-immunodetection.

Glypican

Glypican 1 (GPC1), a membrane anchoring protein, found to be overexpressed in various cancers. GPC1 is highly expressed as assessed by immunohistochemical assessment, in pancreatic cancer tissue as compared to normal tissue.[28] Additionally, GPC1 had an independent prognostic effect on overall survival.[28] Similar results were reported for Glypican 3 (GPC3) in pancreatic cancers. A recent study by Yao et.al reported overexpression of GPC3 associated with progression, carcinogenesis and poor progression in PDC.[29] In a novel approach GPC1 circulating exosomes (GPC1 crExos) were monitored with flow cytometry in serum of patients and mice with cancer by Melo *et al.*[30] GPC1 crExos demonstrated nearly perfect values when comparing patients with PDAC, chronic pancreatitis and healthy individuals. GPC1+ crExos showed a sensitivity and specificity of 100% in each stage of pancreas cancer.[30] Moreover, GPC1+ crExos demonstrated superior prognostic indicator to CA19-9 and elevated levels prior to MRI detectable lesions in pancreatic cancer. These evidences suggest its utility as novel, non-invasive biomarker in early diagnosis and potential use in pancreatic cancer progression.

KRAS mutation

KRAS mutations occur very frequently in pancreatic cancer and were extensively studied. The diagnostic accuracy of KRAS mutation was not optimal for diagnostic utility due to non-specificity of these mutations.[31] The low level of cell-free circulating tumor DNA (ctDNA) in serum limits non- invasive assessments.[32]

Osteopontin

Osteopontin (OPN), a protein of extra-cellular matrix, has been reported to be upregulated in pancreatic cancers with sensitivity of 80% and specificity of 97% for detection of pancreatic cancers.[33] However, the OPN did not diagnostic accuracy over CA 19-9 levels alone, but a diagnostic panel including OPN,TIMP-1 and CA 19-9 achieved better sensitivity and specificity.[34,35] These biomarkers require further investigation to determine their role as a diagnostic biomarker in pancreatic cancer.

Epigenetic markers

Epigenetic changes can contribute to both cancer initiation and progression in PDC that evolves through non-invasive precursor pancreatic intraepithelial neoplasias (PanINs).[36] The PanINs typically take 10 to 15 years to develop into malignant lesions that can further metastasize,[37] thus an ideal context for the early detection. The serum markers, CA19-9 and radiological imaging are not reliable and therefore epigenetically silenced genes such as NTPX2, SARP2, RPRM, and LHX1 are currently under investigation.[38] The sources for assessment of methylation markers include pancreatic juice, cell free tumor DNA and brush samples.[39]

Pancreatic juice with exfoliated cells from diffuse areas of the pancreas, can form a good source for early detection in pancreatic epithelial changes. Emerging data suggests that patients with malignant transformation can be differentiated from benign changes with higher specificity by assessing methylation of the genes: CCND2, TFPI2, PENK, NPTX2, FOXE1, CD1D, KCNK12, CLEC11A, NDRG4, IKZF1, and PKRCB.[40,41] Prediction models generated by assessing methylation promoters of BMP3, RASSF1A, BNC1, MESTv2, TFPI2, APC, SFRP1 and SFRP2 in cell free DNA in plasma generated detection probability with >75% sensitivity and >80% specificity for PDC.[42] Methylation levels of TFPI2, NPTX2 and CCND2 in endoscopic biliary brush samples from patients with PDC, correlated with detection in 73% of patients.[43] Currently, no epigenetic biomarker has been approved for detection, however independent validation in large samples are anticipated in future with increased availability of genome wide analysis.

Predictive biomarkers

The advanced pancreatic cancer patients associated with poor

prognosis in spite of available therapeutic options It appears that overall emphasis in identifying predictive biomarkers is relatively low compared to diagnostic markers, likely due to limited therapeutic options.

Gemcitabine markers

Gemcitabine, a nucleoside analog, since its approval in 1996 has been the cornerstone therapy for neo-adjuvant, adjuvant and palliative chemotherapy in pancreatic cancer. It was suggested that the two genes, GSTM1 and ONECU were found to be differentially methylated between responders and the non-responders.[44] Cellular uptake mechanisms are the key to develop gemcitabine toxicity and resistance.[45] The following nucleoside transporters involved in the uptake of this drug have been evaluated for predicting the gemcitabine response.[46]

Human equilibrate nucleoside transporter 1 (hENT1)

hENT1 relationship with gemcitabine as a predictive biomarker was initially evaluated by immunostaining in a small study that demonstrated significantly longer survival with gemcitabine chemotherapy as compared to without detectable hENT1 (13 months versus 4 months, P=0.001).[47] These findings were validated in a larger cohort and in adjuvant setting validating that hENT1 expression can predict the gemcitabine response correlating with improved survival outcomes.[48,49] An ongoing randomized clinical trial, evaluating if hENT1 can predict response to gemcitabine treatment and whether combination therapy of 5-FU, leucovorin and oxaliplatin (FOLFOX) might be a superior treatment instead of gemcitabine in patients with low hENT1 expression (NCT01586611). Locally advanced unresectable and distantly metastatic PDC are treated with multiagent systemic chemotherapy, combination therapy of folinic acid, 5-FU, irinotecan and oxaliplatin (FOLFIRINOX) that showed longer OS and PFS with FOLFIRINOX as compared to single agent gemcitabine regardless of hENT1 expression. Interestingly, in HENT1 positive patients no significant differences were noted in between gemcitabine alone or FOLFIRINOX.[50] Additionally, patients positive for hENT1 expression in curative resection specimens had better prognosis compared to hENT1 negative patients.[51] Thus, prognostic potential to select subgroup for surgical management.

Human equilibrate nucleoside transporter 1 (hENT1)

hCNT1 and hCNT3 are the major gemcitabine transporters in hCNT group.[52] Higher hCNT3 expression on tumor blocks from patients treated with adjuvant gemcitabine based chemoradiation associated with higher survival rate at 3-years, 54.6% *vs* 26.1% (P=0.028).[52] Additionally, in a combined analysis, patients with two favorable prognostic factors (hENT1(high)/hCNT3(high) expression) had significantly longer survival than those having one or no favorable prognostic factor.[52] With limited data and lack of prospective trial, further studies are warranted to assess use of hCNT as treatment predictive biomarker.

FOLFIRINOX markers

In metastatic pancreatic cancer, FOLFIRINOX (combination of folinic acid, 5-FU, irinotecan and oxaliplatin) reported to have survival advantage as compared to gemcitabine alone.[53] Predictive biomarkers are essential for FOLFIRINOX therapy to avoid unfavorable side-effect profile. Higher tissue CES2 expression was correlated with longer OS and PFS who received neoadjuvant FOLFIRINOX treatment.[54]

Nab-paclitaxel markers

In metastatic pancreatic cancers, combination therapy with albumin based nab-paclitaxel and gemcitabine reported significant improvement in OS and PFS compared to Gemcitabine alone.[55] Glycoprotein osteonectin, also known as secreted protein acidic and rich in cysteine (SPARC), identified as a frequent site for aberrant methylation in pancreatic cancer.[56] Several studies described the role of SPARC overexpression in pancreatic cancer and suggested its role in enhancement of paclitaxel delivery into the tumor as well. This was further clinically evaluated in phase I/II trial, that demonstrated high-SPARC group compared to low-SPARC group, was associated with improved median OS (17.8 months *vs* 8.1 months respectively).[57,58] However, these findings were not validated in phase III study in metastatic pancreatic cancer treated with nab-paclitaxel and gemcitabine.[59] To date, no other reliable marker is reported for nab-paclitaxel therapy in pancreatic cancer patients.

Stromal markers

PDC is quite unique because of extensive fibrosis that surrounds cancer cells, this fibrosis along with a poor blood supply has been found to limit delivery of drugs into cancer cells. A dense desmoplastic stroma surrounding the PDC can cause physical barrier to the delivery of chemotherapy[60] and develop hypoxic tumor microenvironment that is immunosuppressive in nature. This is one mechanism by which pancreatic cancer is resistant to our current standard treatment. Hyaluronan is a major component of the extracellular matrix that comprises the stromal components of PDC and recently emerged as novel therapeutic target. Hyaluronidase is an enzyme that degrades this hyaluronan. The recombinant pegylated form of hyaluronidase (PEGPH 20) has been shown to improve clinical outcomes by stromal depletion leading to tumor vasculature expansion and improvement in drug delivery. This was investigated in the phase 2 HALO-202 study and reported improved median progression-free survival in the PDC patients receiving PEGPH20 with gemcitabine and nab-paclitaxel versus gemcitabine and nab-paclitaxel alone.[61] In the secondary endpoint analysis of patients with high levels of hyaluronan, the median PFS increased to 9.2 months from 5.2 months in favor of combination PEGPH20 and chemotherapy. Therefore, hyaluronan-high status has been considered as a potential predictive biomarker of benefit of PEGPH20 in PDC.[61] The combination of hyaluronidase with immunotherapies is currently under validation in a phase 3 trial.[62]

BRCA mutated tumors

About 4-10% of pancreatic cancer patient are believed to have hereditary predisposition. Patients with familial history of pancreatic cancer, BRCA mutation prevalence can be up to 17%.[63] Inactivation of BRCA1 and BRCA2, PALB2 a subset of tumors may predict response to platinum-based treatments (oxaliplatin, cisplatin and carboplatin).[64] BRCA mutations are a potential predictive biomarker of response to PARP inhibitors and platinum-based chemotherapies. Superior overall survival was reported in stage III-IV pancreatic cancer patients having BRCA mutations treated with platinum (22 month *vs* non-platinum (9 months) chemotherapies.[65] PARP inhibitors are pharmacologic inhibitor of poly(ADP-ribose) polymerase enzymes, studied as potential chemotherapeutic agents in breast and ovarian cancers. Tumors with germ line mutations in DNA repair genes are susceptible to PARP inhibitors. Olaparib, an oral PARP inhibitor was given in a phase II multi centered trial in advanced cancers harboring germline BRCA1/2 mutations. A total of 298 patients received treatments, 23 of whom had pancreatic cancer. Tumor response rate was 21.7% and stable disease >8 weeks was reported in 35% cases of pancreatic cancer.[66] There are several trials currently evaluating PARP inhibitor effectiveness in patients with pancreatic cancers and BRCA mutations.[67]

Microsatellite instability

The reported incidence rate of microsatellite instability (MSI) in PDCs has been variable ranging from <5% to 13–17% of PDC patients.[68,69] While outcomes from single agent immunotherapy trials in PC was disappointing, results from the pivotal KEYNOTE study revealed that pembrolizumab demonstrated 83% objective response rate (ORR) in the six evaluable pancreatic cancer patients[70] suggesting that MSI status can predict the benefit from anti-PD-1/PD-L1 blockade C. It is recommended now to test all PCs for MSI status.

PD-1/PD-L1

Pancreatic cancers are typically deficient in T-cell infiltration which may explain the poor response to single agent immunotherapeutic.[71] PD-L1 overexpression is associated with worse prognosis in a range of solid tumors, including PDAC.[72] PD-L1 expression has been evaluated as a predictive biomarker for response to PD-1 inhibitors in other tumor types and was found to be correlated with better outcomes with PD-1/PD-L1 blockade;[73] however, the relative lack of response to single agent checkpoint inhibitors in PC precludes assessment of PD-L1 as a predictive marker. Several novel vaccine therapies are under investigation to induce T-cell responses and overcome the immune-resistance in PC. IL17 expression in the tumor microenvironment is currently being evaluated as a biomarker for vaccine induced anti-tumor response (NCT02451982).

Prognostic markers

CA 19-9

Ca19-9 has also been studied for its prognostic value. Berardi et.al, reported high levels of Ca 19-9 independent unfavorable prognostic factor. Median overall survival if CA19-9 ≤37 U/mL *vs* >37 U/mL was 18.49 vs. 9.21 months respectively.[74] These findings were validated in other study and reported post-operative decrease in CA 19-9 and post-op value less than 200 U/mL of CA 19-9 predicts improved survival outcomes.[75]

SMAD4

SMAD4 signal transformer from transforming growth factor-beta (TGF-β), involves in pancreatic cells proliferation, apoptosis and serve as tumor suppressor gene. It was reported to be inactivated in more than 50% pancreatic cancer cases.[76] Loss of SMAD4 expression was correlated with distant metastasis. The prognostic role is conflicting with few reports on worse prognosis with loss of SMAD3 expression while few other studies could not confirm those findings.[77,78] To clarify further, Shugang et.al reported prognostic value of SMAD4 in a recent meta-analysis with 14 studies demonstrated the worse prognosis with loss of SMAD4 expression.[79] It is hypothesized that patients with intact SMAD 4 expression associated with relatively less distant progression and therefore local treatment with radiation could improve clinical outcomes. Its value as prognostic biomarkers is currently being validated in the ongoing Radiation Therapy Oncology Trial (RTOG) 1201 trial will further evaluate response of therapy to SMAD 4 status in locally advanced pancreatic cancer patients.[80]

Angiogenesis markers

Stromal cells in pancreatic cancer contribute in tumor progression by releasing angiogenesis factors such as platelet-derived growth factors (PDGF), matrix metalloproteinases (MMPs) and vascular endothelial growth factors (VEGF). Higher tissue and serum levels of these angiogenic markers were correlated strongly with better survival.[81]

Inflammatory markers

Cancer cells activate systemic inflammation pathways which anticipate tumor progression via complicated route involving cancer cell proliferation, inducing angiogenesis, evading growth suppressors and activation of metastasis. Several inflammatory biomarkers have been proposed to predict prognosis in various cancer such as C-reactive protein, platelet to lymphocyte ratio, neutrophil to lymphocyte ratio (NLR)[82] and modified Glasgow prognostic score.[83] Among these, neutrophil to lymphocyte (NLR) has been shown to most valuable in predicting prognosis. NLR >5 appears to indicate shorter OS and poor prognosis in pancreatic cancer.[84]

Immune markers

Several immune markers were investigated immunohistochemically (IHC) markers and correlated with prognosis. IHC markers associated with a worse prognosis include FOXP3, CD68, CD163, CD204, and CD66b;[85] and the markers associated with an improved prognosis include CD3, CD8, CD4, CD20.[86] High CD4+/CD8+ tumor infiltrating lymphocytes following curative resection was found to be an independent favorable prognostic factor for overall survival.[87] The presence of intra-tumoral tertiary lymphoid organs (lymphoid follicles), was associated with longer survival.[88] However the data is conflicting as Hart *et al.* scored intra-tumoral lymphocytes as high or low and found no survival difference.[89] This needs to be investigated in the context of a larger clinical trial. Emerging data also suggests that neoadjuvant therapy may selectively modulate immunosuppressive cells. In a retrospective analysis, significantly lower numbers of Tregs (T-regulatory cells) were identified in resected PDC specimens following neoadjuvant therapy compared with resected tumor specimens from untreated patients.[90] This is likely due to adaption of the immune infiltrate, or by the immune reactivation by neoadjuvant therapy. Whether this activation could be further harnessed by concurrent or sequential administration of immunotherapies is currently under investigation.

Micro RNA's

Beside its role as diagnostic biomarker, miRNAs, have been evaluated as potential prognostic marker. In a recent meta-analysis by Frampton et.al, demonstrated decreased OS and disease-free survival (DFS) in patients expressing high miR-21, miR-155 and miR-203; and low miR-34a levels.[91] Other studies demonstrated that lower expression of miR-494 and miR-218 and high miR-221 and miR-744 levels predict poor prognosis in pancreatic cancer.[92-95]

SPARC

SPARC as discussed above as predictive biomarker for nab-paclitaxel, was evaluated for prognostication. SPARC overexpression in pancreatic cancer indicate poor outcome.[96] Interestingly, SPARC overexpression in pancreatic cancer stromal cell demonstrated poor prognosis but its expression in tumor cells was not associated with prognosis.[97] Comparable results were reported for SAPRC mRNA expression in tissues of PDC patients.[98]

Challenges in biomarkers studies

One of the major challenges in biomarker development is the collection of tumor tissue of adequate quality for analysis. Early diagnosis of PDC is usually performed with fine needle aspiration and therefore adequate tissue procurement is difficult to

obtain. Pre-chemotherapy tumor biopsies frequently contain limited tumor cells (15%) or did not have ≥50% tumor content for high-quality tissue assessments.[99] The failure in finding high-sensitive and high-specific biomarkers may also be attributed to the availability of relative fewer samples and lack of proper matching with cases and controls. It is also challenged by inadequate standard operating procedures in terms of sample collection, storage, analysis and interpretation of results. The antibody-based technologies for biomarker discovery have been challenged by lack of robustness, and relatively low throughput requiring multiplexing and complex assay validations.[100] These in general also associated with high costs and requirement of large volume of samples

Conclusions

A diverse array of novel biomarkers in terms of their diagnostic, predictive and prognostic potentials are currently being studied with the hope of finding effective management for this challenging cancer. Studies on innovative molecular markers such Glypican-1 and micro-RNA's, yielded encouraging results. The emerging immunomodulatory treatments for PDC present an opportunity for predictive biomarker development. Various combinations of these biomarkers demonstrated their potential use. However, the biomarker studies have been challenged by relatively low case numbers, absence of feasibility studies only, selection of early stage samples and non-specificity of molecular markers. Nevertheless, large studies with novel study designs are warranted to validate these biomarkers for clinical application. The optimal therapeutic management should be guided by the molecular composition of their tumor and these biomarkers play a crucial role in defining the way for precision treatment.

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