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Contents

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Emerging role of non-invasive and liquid biopsy biomarkers in pancreatic cancer

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Abstract

A global increase in the incidence of pancreatic cancer (PanCa) presents a major concern and health burden. The traditional tissue-based diagnostic techniques provided a major way forward for molecular diagnostics; however, they face limitations based on diagnosis-associated difficulties and concerns surrounding tissue availability in the clinical setting. Late disease development with asymptomatic behavior is a drawback in the case of existing diagnostic procedures. The capability of cell free markers in discriminating PanCa from autoimmune pancreatitis and chronic pancreatitis along with other precancerous lesions can be a boon to clinicians. Early-stage diagnosis of PanCa can be achieved only if these biomarkers specifically discriminate the non-carcinogenic disease stage from malignancy with respect to tumor stages. In this review, we comprehensively described the non-invasive disease detection approaches and why these approaches are gaining popularity for their early-stage diagnostic capability and associated clinical feasibility.

Core Tip: Considering the limited commercial availability of cell free nucleic acids and secreted proteome-based non-invasive biomarkers it is crucial to summarize them for the proper diagnosis of pancreatic cancer and distinguish it from other benign pancreatic diseases. We also highlighted the clinical use of these non-invasive biomarkers in diagnostics. This review will successfully guide readers to address the current issues and aid in the cutting-edge development of biomarkers with higher sensitivity and specificity.

INTRODUCTION

The global population is severely burdened by the increase in multiple types of cancer. Cancer-related incidence and its associated mortality have emerged as a major threat to global health in developed countries, like the United States, European countries, Canada, and Australia, and developing as well as under-developed countries including India and Southeast Asian countries. Pancreatic cancer (PanCa) is a deadly and emerging type of cancer because of its associated poor survival. Only 20% of patients are eligible for surgery due to the physiologically deep-rooted location and the generalized symptoms caused by the close proximity to crucial vessels. In 2022, the estimated new incidences in the United States alone are 62210 (male) and 32970 (female), and the estimated deaths associated with PanCa are 25970 males and 23860 females.

Per the GLOBACON 2020 data, PanCa is the 12th most common cause of cancer with 495000 new cases across the globe with approximately 47% of the new cases registered in Asia and another major proportion of 28% in Europe. There might be an increase in the incidence by 70% by the end of 2022, which translates into approximately 844000 new cases per year. As documented in the United States population, PanCa-related mortality has relatively increased in men than in women. In the United Kingdom, PanCa is the tenth most frequent cancer type, and it ranks fifth in terms of cancer-related deaths per year. Some risk factors associated with PanCa apart from genetic predisposition include obesity, diet and smoking. PanCa-associated surgery has a high mortality risk making it a more life-threatening disease[1-4]. Based on the demography and ethnicity of patients, the profile of biomarkers and predisposition to particular risk factors can vary[4,5].

LIQUID BIOPSY AND ITS ROLE IN PANCA DIAGNOSIS

Liquid biopsy (also known as a fluid biopsy) is an approach to identify markers released from tumors in the blood or associated body fluids. It includes but is not limited to cell-free DNA (cfDNA), cell-free RNA (cfRNA), cell-free proteins, circulating tumor cells and exosomes[6-8]. Liquid biopsy is rapidly developing and has a highly emerging market with an estimated $5 billion (USD) turnover globally by 2023[9]. DNA is the most dominant approach in terms of biomarker identification from plasma or serum and in terms of availability for clinical practices, which makes cfDNA a lucrative option.

There are multiple factors that make liquid biopsy an attractive option in comparison to tissue biopsy. A major reason is that traditional tissue biopsy fails to identify tissue heterogeneity in depth whereas liquid biopsy does that more efficiently[10]. The reason behind this is based on the concept that various categories of tumor materials are released in the blood irrespective of tumor heterogeneity. Therefore, making the liquid biopsy samples, such as plasma or serum, a cocktail that has tumor markers irrespective of cell type is not present in the case of the traditional biopsy technique. In addition, the exponential growth of next generation sequencing technologies enables the identification of cell free nucleic acids with high sensitivity, which is of great clinical application.

Moreover, traditional tissue biopsy is not applicable in the case of patients that are in an early stage and have not developed a tumor of significant size[11]. Early-stage PanCa is usually asymptomatic, and no operative options are available to examine the tumor, making traditional tissue biopsy non-

functional in this context. For early or advanced diagnosis and disease assessment, liquid biopsy can be a major step forward toward cancer prevention and screening of populations. APAPTEST was the first cancer blood-based test done to identify markers in the case of cervical cancer[12]. Disease-targeted liquid biopsy-based biomarkers can detect patients who have an intermediate chance of developing PanCa such as those having chronic pancreatitis (CP), diabetes or a genetic predisposition to PanCa. It can also distinguish between PanCa and pancreatic diseases that are immune system modulated in their origin.

A crucial requirement is distinguishing autoimmune pancreatitis (AIP) from PanCa. AIP diagnosis, which is an autoimmune disease based on the rise in IgG4 levels in serum, is based on a liquid biopsy-based approach for identification and differentiation from malignancies such as PanCa. Upregulation of IgG4-positive plasma cells in organ tissues followed by increased IgG4 levels in the serum is usually defined as type 1 AIP. On the contrary, type 2 AIP has no connection with IgG4. It demonstrates features in the pancreatic ducts resembling granulocytic epithelial lesions. Type 1 AIP also acts as a pancreatic lesion related to IgG4-related disease[13]. Since both type I AIP and type II AIP are based on identification using non-invasive approaches, the development of these techniques is urgent to support cases where invasive approaches are a less viable option. This approach acts as a powerful alternative to existing diagnostic methods and will be highly beneficial for the identification of cases with aggressive tumor biology and support clinicians to make proper individualized targeted therapy[13-17].

The methylation status alteration in cancer cells is also an important point to consider for disease diagnosis. The nature of methylation based alterations discriminates itself from genetic changes based on tumor tissue type specificity, which is crucial in the case of methylation alterations apart from being more pervasive and prevalent in terms of occurrences[18,19]. Aberrant DNA methylation alterations mostly occur in CpG islands and play a vital role in activating tumor suppressor genes and oncogenes, thereby modulating the genome[20-23]. The approach to identifying cfDNA-based methylation changes is a growing area of interest because invasive approaches are not always feasible and proactive in terms of clinical diagnostics[24,25]. Various reports claimed that in the case of pancreatic ductal adenocarcinoma (PDAC) an altered methylation signature has been observed when tumor tissues were compared with normal tissues, hence directing its implications in biomarker development[26,27].

The neoplastic tendency of the pancreas needs to be identified, and specific non-invasive markers discriminating PanCa from CP or other pancreatic precursor lesions such as pancreatic intraepithelial neoplasia (PanIN), intrapapillary mucinous neoplasia (IPMN) and mucinous cystic neoplasm need to be developed[28]. Luo *et al*[29] documented that cg10673833, a circulating tumor DNA (ctDNA) methylation marker was found to be very sensitive (89.7%) and specific (86.8%) for the detection of colorectal cancer and its associated pre-cancerous lesions. SEPT9 was the first blood-based screening test that the Food and Drug Administration approved for colorectal cancer. Cancer-seek was developed by Tivey *et al*[30]. It is a blood test that detects circulatory protein and cfDNA biomarkers. It is capable of detecting eight cancer types, mostly in their respective advanced stages, with a specificity of 99% and a sensitivity of 69%-98%, which varied based on cancer type[12,20].

PANCA AND ITS ASSOCIATED BENIGN DISEASES

Systemic approaches to develop biomarkers to distinguish PDAC from other precancerous lesions are of great importance as they will also support the early diagnosis of PDAC and substantially improve outcomes. Single gene analysis has its limitation in early detection because it has been observed that the specificity and sensitivity of biomarkers in combination increase more than single biomarkers. Traditional serum-based biomarkers like CA19-9, CA125, and carcinoembryonic antigen (CEA) for PanCa diagnostics are of immense importance, but there is a need for the development of more sensitive and specific non-invasive biomarkers. The sensitivity and specificity of detection are usually much higher when the above three biomarkers are used jointly instead of individually[21,31]. In addition, varied combinations of DNA methylation-based biomarkers are being developed to better understand the complexity of the diseases including both the initiation and neoplastic stages. Concurrently addressing the contribution of immune cells in the cfDNA pool and its associated methylation or hydroxyl-methylation pattern is of great importance as it can be obtained from plasma samples[32-35].

Almost 50 of 100000 people are affected by CP worldwide with an increasing trend, and a CP-associated prolonged inflammatory state enhances tissue conversion to neoplasia. Pancreatic tumor (PT) formation is also increasing along with CP worldwide. Mortality due to PTs is close to 6%. After a diagnosis of CP, it takes almost 10 years before tumor symptoms appear, which is a long latency period with sufficient time for early diagnosis and treatment *via* non-invasive approaches. *PENK* DNA methylation has been observed broadly in precancerous lesions of various grades including extraductal and intraductal PTs and CP along with PanIN, IPMN and mucinous cystic neoplasm. *PENK* methylation alterations increased as the neoplastic tissue grade increased but was absent in the case of AIP and adjacent normal pancreatic tissue[36].

In a study of an 87 gene panel, 6 genes, namely *PRKC, IKZF1, CD1D, KCNK12, CLEC11A* and *NDRG4*, were shown to have clinical ability and potential as a cfDNA diagnostic biomarker for PanCa[37]. In this

study using pancreatic juice taken from PanCa, CP and healthy patients, the authors documented that most of the biomarkers were successful in discriminating patients with PanCa from healthy patients, but only *CD1D* was successful in discriminating patients with CP from PanCa with a sensitivity of 84%[37, 38]. A remarkable change in methylation status was documented by Liggett *et al*[39], where they showcased that hypomethylated genes in CP converted to hypermethylated genes in PanCa, suggesting that altered methylation is also a driving reason for neoplasia development in the case of PTs.

A total of 14 genes, namely *CCND2, CDKN2B, DAPK1, MUC2, MYOD1*, promoter A of *MLH1, CDKN1C, ESR1, MGMT PGK1*, the proximal region of the *PGR* promoter, *RARB, RB1* and *SYK*, were successful in distinguishing CP from PanCa based on cfDNA DNA methylation profiling with 91% sensitivity and specificity[38,39]. In a study to identify cell death specific to tissue type by Lehmann-Werman *et al*[40], they observed that *CUX2* and *REG1A* are specific to pancreas tissue type. They also found these genes to be hypomethylated in plasma in both PanCa and CP, but the levels were lowered in other lower neoplastic grade lesions. *CUX2* was more hypomethylated in the case of PanCa and *REG1A* was higher in CP, which gives the ability to discriminate CP from PanCa based on tissue death irrespective of etiology[38,40].

In a study using pancreatic juice samples collected from PanCa and other associated precancerous lesions, six genes were demonstrated to be differentially methylated between CP and PanCa[41]. The six genes, *TFPI2, NPTX2, FOXE1, p16, ppENK* and *CyclinD2*, were able to successfully distinguish between CP and PanCa as CP had lower levels of methylation than PanCa. Similarly, the levels differed amongst healthy tissue and high-risk patients creating a gradient of altered methylation across various stages starting from healthy to precancerous to PanCa samples. It was also able to distinguish between IPMN and PanCa based on cfDNA methylation from pancreatic juice samples. Further, the study also attempted to address the limitations of IPMN diagnostics, which cytology mostly fails[42]. Promoter DNA methylation of the *CDO1* gene was identified to be involved in early-stage PanCa based on cytological experiments. cfDNA methylation analysis of *CDO1* from PanCa, CP and AIP patients showed that significant promoter methylation was associated with PanCa but was absent in CP and AIP [43]. In another study, *CD1D* showed cfDNA methylation when analyzed in pancreatic juice samples of PanCa [area under the curve (AUC): 0.92], but no methylation was documented in benign diseases[21, 43]. In a study by Humeau *et al*[44], they reported that urine and sputum are also suitable alternatives to plasma for cell-free nucleic acid biomarker development. The utility of other material such as urine as a source of non-invasive testing needs further data and investigation.

MicroRNA (miRNA), namely hsa-miR-23a and hsa-miR-23b, have been documented to be overexpressed in IPMN patient saliva, which makes it a suitable precancerous lesion biomarker. It was also observed that hsa-miR-210 along with let-7c signals may be leveraged from saliva in distinguishing CP from healthy subjects[32,44]. Su *et al*[45] concurrently conducted a study using serum as a sample source and observed that miR-877, miR-3201, miR-890, miR-602 and miR-16-2-3p were successful in distinguishing low-risk and high-risk PDAC[32,45]. Irrespective of this development, there is still a hindrance in using miRNAs in clinical diagnostics due to reproducibility issues and variable expression of miRNAs. However, substantial work is ongoing in this area[32].

In a study to differentiate PDAC from CP by Bosch *et al*[46], it was documented that the the presence of a high level of soluble AXL (sAXL) was associated with lower overall survival in the case of PDAC. When identified from plasma, a higher level of sAXL was documented in PDAC than in CP and healthy controls, and they concluded that it is a potential biomarker for discriminating CP from PDAC and useful in identifying the onset of PDAC by its increasing level during monitoring. The findings were also validated in murine models. sAXL was also found to be overexpressed in IPMN, which is a pre-PDAC stage[46].

Weeks *et al*[47] conducted research where they studied the urine proteome differences amongst PDAC, CP and healthy individuals. They noticed 101 proteins that were significantly altered in PanCa and CP. S100A9 was found to be downregulated in PanCa but overexpressed in CP. Annexin A2, CD59 and gelsolin proteins were also found to be dysregulated in PDAC samples[47]. An isotope-coded affinity tag tended to successfully distinguish between PanCa and CP based on quantitative protein expression[41]. In this review, we documented the important cell free biomarkers that are vital in the early-stage detection of PanCa and are able to distinguish PanCa from its non-malignant precursors.

CFDNA AND ITS ASSOCIATED SECRETED PROTEOME

cfDNA in PanCa and its association with benign pancreatic diseases

First reported in 1948 by Mandel and Meta[48], cfDNA is undoubtedly the most dominant form among the liquid biopsy-based approaches and when released by tumor is also a ctDNA. Leon *et al*[49] in 1977 introduced cfDNA levels, which were found to be notably more advanced in malignancies than in healthy individuals thereby showcasing its role in carcinogenesis. Similar observations were made in 1983 with PDAC patients, and it was noted that cfDNA levels were higher in PDAC patients than in CP and acute pancreatitis[50]. The origin of cfDNA in the blood can be due to release from tumors, apoptosis or necrosis and other active cellular secretions. A major factor leading to the rise in cfDNA

studies in cancer management is the half-life of cfDNA, which is around 16 min-2.5 h, and that it can be monitored for overall disease progression and associated clinical prognosis with medical treatment (Table 1)[12,49,51].

Cancer diagnosis and treatment face a major setback from recurrence of the malignancy that cannot be ideally monitored using traditional techniques due to the lack of proper tumor formation and its associated diagnostic techniques. Postoperative or post-chemotherapeutic analysis of ctDNA in the blood can also be referred to as minimal residual disease (MRD) analysis. Minimal residual disease analysis cannot be ideally performed using imaging and traditional biopsy techniques, and the analysis using cfDNA/ctDNA is a great advance to measure tumor recurrence (Table 1)[12,52].

In terms of aberrant DNA methylation, which is also a common observation in PDAC patients, it has been documented that a change in methylation levels has been observed in various stages of dysplasia [53,54]. This indicates that the level of cfDNA methylation across various pre-PanCa lesions to PDAC is altered providing a candidate opportunity to track PDAC progression. In 2009, Melnikov *et al*[55] established the use of cfDNA methylation in PDAC diagnosis from 30 plasma samples. This was completed using microarray technology where they identified that the promoter region was hypomethylated in five genes with a sensitivity and specificity of 76% and 59%, respectively[55]. A crucial step in PanCa treatment is staging as that provides a proper clinical treatment approach and is associated with DNA promoter hypermethylation. Henriksen *et al*[56] reported that the use of cfDNA promoter hypermethylation as a prognostic biomarker in PDAC staging, which is based on the hypothesis that cfDNA methylation is stage-specific, can help in stage classification. Henriksen *et al*[56] analyzed a set of 28 genes in PDAC plasma samples. They developed the first prognostic prediction model using cfDNA hypermethylation targeting PDAC staging. They separated cluster cases with and without distant metastasis. The researchers were also able to document significantly higher numbers of hypermethylated genes clustering in stage IV PDAC than in earlier stages amongst which *BNC1* was the most significant gene of interest (Table 1)[25,27,56].

A crucial challenge in cell free nucleosome identification for the differentiation of benign pancreatic disease from PanCa is a nonspecific rise of cell free nucleosome levels in the serum of patients while quantifying the cell free nucleosome. Bauden *et al*[36] identified the cell free nucleosome epigenetic alterations specific to disease type and stages by isolating intact nucleosomes and then quantifying them using an enzyme-linked immunosorbent assay. They were successful in quantifying five serum cell free nucleosome diagnostic biomarkers with an AUC of 0.95, which differentiated PanCa from a healthy pancreas. The epigenetic profiles of cell free nucleosomes often differ between individual patient serum samples based on cancer and healthy populations. Understanding that the epigenetic changes usually occur at the onset of the neoplastic transformation stages, those already in pre-neoplastic stages may represent cell free nucleosome profiles that can act as possible cell free biomarkers for the early detection of cancer (Table 1)[36].

Role of cfDNA as a diagnostic and therapeutic biomarker

Tumors in the pancreas can occur in tissues that are hard to isolate due to their depth of location and also small tumor size can be a limitation. Therefore, in this context blood-based cell free biomarkers are of high clinical importance[56]. Melnikov *et al*[55] investigated that they developed the MethDet56 test which contained screening of 56 gene promoters from PDAC plasma samples that identified five promoters namely *CCND2, SOCS1, THBS1, PLAU* and *VHL* with significant aberrant DNA methylation and with sensitivity and specificity of 76% and 59% respectively. These are initial biomarker development work showcasing that blood-based biomarkers could be highly valuable in diagnosis and prognosis if further studied and developed[55]. In a study by Sato *et al*[57], three out of six genes, namely *CLDN5, SFRP1* and *NPTX2,* previously validated in tissue samples were found to be differentially methylated in pancreatic juice samples collected from PTs[38,57]. However, Matsubayashi *et al*[42] concluded that five genes that were previously validated by solid biopsy failed were not replicated in liquid biopsy. Although the genes *TFPI2*, *PENK, CCND2, NPTX2* and *FOXE1* were successfully able to discriminate PanCa from healthy tissue, the analysis failed to discriminate CP from PanCa or healthy tissue based on cfDNA methylation (Table 1)[42,55].

Yi *et al*[58] interestingly found the use of *BNC1* and *ADAMTS1* as novel serum-based biomarkers for early PanCa detection. The same research group concluded that low frequencies of these two genes were observed in pancreatitis, and there was a significant methylation difference between PanINs and PanCa. *BNC1* could still be detected in PanIN-1, but *ADAMTS1* could only be detected significantly in invasive PanCas[58]. Li *et al*[59] reported in an analogous study that *BNC1* and *SEPT9* were found to be highly methylated in PanCa and were suggested to have a significant role in PanCa diagnostic cfDNA biomarker development from plasma. A limitation of the study was that cfDNA methylation level in plasma was also found to be elevated in other benign diseases such as CP, acute pancreatitis and other lesions as these are risk factors leading to PT formation. This suggests a possible role of methylation changes in driving the progression of benign lesions to metastatic PanCa[60].

Global epigenetic alterations in 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) work as standalone entities in carcinogenesis[61]. Cao *et al*[62] concluded in this context that 5mC as well as 5hmC work as effective cfDNA in PDAC diagnostics and that when 5mC-5hmC work as an integrated system it provides an enhanced diagnostic power. As reported by Guler *et al*[33], PanCa early detection

Table 1 Cell free DNA involved in pancreatic cancer diagnostic and prognostics			
Target candidate	Up/down	Sample	Potential value
CEA mRNA	Up	Whole blood	D
EGFR mRNA	Up	Serum	D
4GnT mRNA	Up	Whole blood	D
COL6A3 mRNA	Up	Serum	D/P
Mir-155	Up	Plasma	D
miR-196a	Up	Plasma	D
miR-21	Up	Plasma	D
miR-210	Up	Plasma	D
miR-155	Up	Pancreatic juice	D
miR-21	Up	Plasma	D
miR-196a	Up	Serum	D/P
miR-200a	Up	Serum	D
miR-200b	Up	Plasma	D
miR-210	Up	Plasma	D
miR-18a	Up	Plasma	D/T
miR-16	Up	Plasma	D
miR-196a	Up	Plasma	D
miR-185	Up	Serum	D
miR-191	Up	Serum	D
miR-20a	Up	Serum	D
miR-21	Up	Serum	D/P
miR-24	Up	Serum	D
miR-25	Up	Serum	D
miR-99a	Up	Serum	D
miR-1290	Up	Serum	D
miR-221	Up	Plasma	D/T/P
miR-375	Down	Plasma	D/T/P
miR-375	Up	Plasma	D
miR-27a3p	Up	Whole blood	D
miR-196a	Up	Serum	D
miR-196b	Up	Serum	D
miR-205	Up	Pancreatic juice	D/P
miR-210	Up	Saliva	D/P
miR-492	Up	Plasma	D/P
miR-1427	Up	Plasma	D/P
miR-22	Up	Plasma	D
miR-642b	Up	Plasma	D
miR-885-5p	Up	Plasma	D
Multigene index	Up	Whole blood	D
miR-483-3p	Up	Plasma	D
MIR-21	Up	Serum	D

MIR-210	Down	Serum	D
MIR-155	Up	Serum	D
miR-222	Down	Serum	D
miR-203	Down	Serum	D
miR-132	Down	Serum	D
miR-212	Down	Serum	D
miR-96	Down	Serum	D
mi-126	Down	Serum	D
mi-217	Down	Serum	D
Let-7	Down	Serum	D
miR-144	Down	Serum	D
miR-148	Down	Serum	D
miR-34a	Down	Serum	D
miR-3548	Up	Serum	D

CEA: Carcinoembryonic antigen; D: Diagnostic; P: Prognostic; T: Therapeutic; miR: MicroRNA.

is suitable using 5hmC-based detection techniques, and a regression model showed an AUC of 0.919 encompassing a highly variable gene set. The low cfDNA methylation observation in the case of PDAC was consistent with other studies, and it corresponded to *KRAS* mutation increase in malignancy. PDAC 5-hmC increase was observed at the transcription start site and 3' untranslated region, but a decrease was seen in the promoters[33]. cfDNA-based *KRAS* mutation identification is usually a good indicator of neoplasia though with limited specificity. *KRAS* remains a widely used gene for the early stage detection of PanCa. Other gene mutations, such as *DNMT3A, TP53, GNAS, JAK2* and *BCORL1*, remain alternative ctDNA-based biomarkers[24,63-65]. However, it is clear that analysis of somatic genes in ctDNA provides limited sensitivity in detecting early-stage tumors (Table 1).

Cell free protein (secreted proteome) in PanCa and its associated benign pancreatic diseases

The secreted proteome often acts as a suitable diagnostic biomarker, but currently only CA19-9 is a Food and Drug Administration-approved biomarker for PDAC diagnosis. It has substantial drawbacks and lacks a proper proteomic dataset for PanCa. Often false negative CA19-9 results are given by patients with *FUT3* mutations, and false positives are also observed in non-malignant CP patients as CA19-9 is usually very highly overexpressed[46].

The plasma-free amino acids (PFAAs) index is growing in popularity as a suitable approach for the diagnosis of PanCa. A study to compare the PFAAs index between PanCa and healthy controls by Fukutake *et al*[66] found PFAAs as a significant biomarker capable of distinguishing PDAC. The receiver operating curve AUC developed index was demonstrated to suitably discriminate PanCa from controls. PFAAs are modulated by metabolic changes, in particular in organ systems based on disease induction. As a result, most metabolomic studies consider the analysis of the PFAAs index[66]. Sources of protein biomarker analysis are usually blood and pancreatic juice, but it can also be validated from tissue samples. Wu *et al*[21] documented that altered levels of proteins, namely GPC1, PFAA, OPNT + TIMP-1, CPA4, MUC5AC and C4BPA, were observed in the serum of PanCa patients. They also documented that GPC1 level was significantly higher in PanCa patients than in patients with benign pancreatic diseases such as CP or AIP. It was also documented that serum levels of cell free CPA4 were significantly higher in PanCa than in healthy controls with a parallel observation that C4BPA was comparatively more sensitive than CA19-9. A crucial observation was based on PFAA where it was revealed that PFAA levels correlated with PanCa staging and that can be used for pathological PanCa staging. A significantly high level of serine in plasma was also documented along with a significant decrease in threonine levels in PanCa patient plasma (Table 2)[21,66,67].

Proteomic techniques are gaining a lot of attention for studying protein expression in PanCa. Urine as a source for the non-invasive analysis of biomarkers has gained attention. Advantages of using urine are: (1) Common proteins such as albumin are less abundant; (2) Sample processing is simpler (the plasma secreted proteome (secretome) is much more complex than the urine proteome); (3) Urine is more thermostable; and (4) 49% of proteins are soluble products in urine obtained from glomerular filtration of lymphatic fluids (indicates that various external sources contribute towards the pool of the urine proteome and its protein components). Similar studies have been completed in breast and ovarian cancers; and it is likely to remain an active area of investigation (Table 2)[47,68,69].

Target candidate	Up/down	Sample	Target	Pathway	Potential value
Table 2 Cell free protein (secreted proteome) involved in pancreatic cancer diagnostic and prognostics					
ADAMTS1	Up	Plasma	ADAMTS1	Methylation	D
BNC1	Up	Plasma	BNC1	Methylation	D
CDKN1C	Up	Plasma	CDKN1C	Methylation	D
MLH1	Down	Plasma	MLH1	Mutation	D
PGR (prox)	Up	Plasma	PGR	Cell signaling	D
SYK	Up	Plasma	SYK protein, mRNA	Cell signaling	D
CCND2	Up	Plasma	Cyclin D2	Cell signaling	D
ALX4	Up	Plasma	Transcription factor	Mutation	P
APC	Down	Plasma	APC	Mutation	P
BMP3	Up	Plasma	TGFβ	Cell signaling	P
BNC1	Up	Plasma	BNC1	Cell signaling	P
BRCA1	Up	Plasma	BRCA1	Mutation	P
CDKN2B	Up	Plasma	Ink4, p16, p14	Cell signaling	P
CHFR	Up	Plasma	E3 Ubiquitin-protein	Cell signaling, check point	P
ESR1	Up	Plasma	ESR1, Transcription factor	Cell signaling	P
EYA2	Up	Plasma	EYA2	Cell cycle	P
GSTP1	Up	Plasma	GSTP1	Cell signaling	P
HIC1	Up	Plasma	HIC ZBTB TRANSCRIPTIONAL RECEPTOR 1	Cell signaling	P
MEST1v2	Up	Plasma	Alpha/Beta hydrolase superfamily	Cell signaling	P
Foxe1	Down	Pancreatic juice	Foxe1	Methylation	D
MGMT	Up	Plasma	MGMT	Methylation	P
MLH1	Up	Plasma	MLH1	Mutation	P
NPTX2	Up	Pancreatic juice	NP, synaptic proteins	Cell signaling	P
NEUROG1	Up	Plasma	NEUROG1	Cell signaling, regulatory network	P
RARB	Up	Plasma	RARB	Cell signaling	P
RASSF1A	Down	Plasma	RAS effector protein	Methylation	P
SFRP2	Up	Plasma	SFRP2	Wnt signaling	P
SEPT9v2	Up	Plasma	Septin9	Cell cycle	P
CDID	Up	Pancreatic juice	CDID	Methylation	D
PRKC	Up	Pancreatic juice	PRKC	Methylation	D
IKZF1	Up	Pancreatic juice	IKZF1	Methylation	D
KCNK12	Up	Pancreatic juice	KCNK12	Methylation	D
CLEC11A	Up	Pancreatic juice	CLEC11A	Methylation	D/P
NDRG4	Up	Pancreatic juice	*NDRG4* promoter	Methylation	D
SST	Up	Plasma	SST	Downstream signaling, transcriptional regulation	P

TFPI2	Up	Pancreatic juice	TFPI2	Gene silencing, cell adhesion plasmin signaling	P
TAC1	Up	Plasma	TAC1	Gpcr downstream, signal transduction	P
VIM	Up	Plasma	Vimentin	Cell signaling	P
WNT5A	Up	Plasma	WNT5A	Wnt signaling	P
PENK	Up	Pancreatic juice	Proenkephalin	Apoptotic, downstream signaling	
DCC	Down	Plasma	Netrin-1 Receptor	Mutation	D
P16	Down	Pancreatic juice	CDKN2A	Mutation, cell cycle regulation	D
P14	Down	Plasma	CDKN2A	Cell cycle	D
DNMT1	Up	Plasma	DNMT1	Mutation	D
DNMT3A	Up	Plasma	DNMT3A	Methylation	D
DNMT3B	Up	Plasma	DNA	Methylation	D

D: Diagnostic; P: Prognostic.

Role of secretome as a diagnostic and therapeutic biomarker

Cell free nucleic acid alone or combined with the secretome is likely to enhance sensitivity in PanCa diagnosis, and it remains an area of high interest in the field. Research often claims that patients with some signature of circulating proteins ideally overlap with circulating DNA. The main issue is that cfDNA and cell free proteins are most often seen in advanced stages than in early stages[63]. A secretome study to identify cell free proteins as biomarkers in PanCa is usually dominated by the urine proteome. In a study by Poruk *et al*[70], the researchers validated a pair of biomarkers to distinguish between PDAC and healthy controls as well as distinguishing it from CP. This group also focused on the ability of the biomarker to identify the early onset of the disease. The pair of biomarkers were OPN and TIMP-1, and they were found to be uniformly overexpressed in the case of PDAC. Due to their tendency to be available as the secretome in blood, they were easy to detect and were shown to be a useful tool for diagnosis. OPN particularly acts as a more advanced prognostic biomarker by itself[70-72]. Urine samples are also a reliable source of cell free biomarker isolation and identification.

A three-protein biomarker panel was developed from urine samples of PDAC patients and were assayed using GeLC/mass spectroscopy (MS)/MS and validated using an enzyme-linked immunosorbent assay. The candidate protein biomarkers were namely LYVE-1, TFF1 and REG1A, and they provided an AUC of 0.89. These biomarkers were able to distinguish between early-stage PDAC and advanced-stage PDAC and were almost consistent with CA-19 levels that were documented in regard to every PDAC stage. The panel was also able to distinguish between PDAC and CP based on the higher levels of biomarkers in PDAC than in CP as well as PDAC stage I-II. The concentrations of these proteins were significantly higher than CP, which generated a clear demarcation between these groups [73].

Kaur *et al*[74] reported MUC5AC as a suitable biomarker candidate for PanCa diagnosis based on its capability to differentiate between PanCa, CP and AIP. Another urine-based diagnosis study claimed that neutrophil gelatinase-associated lipocalin can provide valuable information on the onset of PanCa identification[21,74]. Considering that pancreatic juice collection is an invasive process, the parallel study was completed studying the role of ARG2 as PanCa biomarker, which was specific in targeting the bile component for PanCa early-stage detection. sLR11, which is present in the bile of PanCa cases, was able to discriminate between PanCa and healthy controls[75]. A panel of CA19-9, CA 242, CA 125 and CEA obtained from serum showed a sensitivity and specificity of 90% and 94%, respectively[76,77]. A novel nucleosome marker panel developed by Bauden *et al*[36] showed better sensitivity than CA19-9 in discriminating PanCa from a healthy control. The markers were *H3K4Me2, H2AZ, H2AK119Ub, H2A1.1* and 5mC[36]. Additionally, as documented by Melo *et al*[78], the absolute prediction (AUC = 1.0) of PanCa using glypican 1 on exosomes identified by MS showed utility as a biomarker (Table 2).

cfRNA in PanCa and its association with benign pancreatic diseases

Amidst the traditional serum-based biomarkers like CA19-9, CA125 and CEA for PanCa diagnostics, there is an immense need for the development of more sensitive and specific non-invasive biomarkers. Sensitivity and specificity are usually higher when the three are used jointly (Table 3)[21,31].

Uncontrolled proliferating cancer has a high mortality rate, and treatment and diagnosis are also difficult due to the lack of screening imaging modalities and specific biomarkers. Emerging evidence of biomarkers generated from tumor cells contains a high quantity of RNA in the bloodstream that block

Table 3 Cell-free RNA involved in pancreatic cancer diagnostic and prognostics

Target candidate	Up/down	Sample	Target	Pathway	Potential value
ERBB2	Up	Plasma	Ch 12, *KRAS*	Mutation	D
CDKN2A	Down	Plasma	Gene, Ch 9p21	Mutation	D
SMAD4	Down	Plasma	TGFβ	Cell signaling	D
TP53	Down	Plasma	P53 protein	Mutation/cell signaling	D
EGFR	Up	Plasma	Exon 19, 21	Cell signaling/mutation	D
PBRM1	Down	Plasma	SWI/SNF ch remodeling comp	Mutation, immune checkpoint blockade	D
KMT2D	Down	Plasma	FOX1-miR-1224, HIF/GATA5-miR-133a	Mutation	D
RNF43	Down	Plasma	RNF43PROTEIN	Wnt signaling	D
TP63	Down	Plasma	P63 protein	Cell signaling	D
MTOR	Up	Plasma	mTOR	mTOR signaling	D
NRAS	Up	Plasma	*NRAS*	Mutation	D
HRAS	Up	Plasma	*HRAS*	Mutation	D
BRCA1	Down	Plasma	BRCA1	Signaling	D
BRCA2	Down	Plasma	BRCA2	Signaling	D
PALB2	Up	Plasma	PALB2	Signaling/mutation	D
GPC1	Up	Serum	MAPK, PI3K-AKT-mTOR, Hedgehog signaling	Cell signaling	D
PFAA	Up	Serum	MAPK, PI3K-AKT-mTOR, Hedgehog signaling	Cell signaling	D
OPNT+TIMP-1	Up	Serum	MAPK, PI3K-AKT-mTOR, signaling	Cell signaling	D
CPA4	Up	Serum	MAPK, mTOR, Hedgehog signaling	Cell signaling	D
MUC5 AC	Up	Urine/pancreatic juice	MAPK, PI3K-AKT-mTOR, Hedgehog signaling	Cell signaling	D/P
C4BPA	Up	Serum	MAPK, PI3K-AKT-mTOR, Hedgehog signaling	Cell signaling	D
OPN	Up	Serum	OPN	Cell signaling	P
TIMP-1	Up	Serum	TIMP-1	Cell signaling	D
NGAL	Up	Urine	Receptor binding protein	Cell signaling	D
ARG2	Down	Pancreatic juice	Beta cell function	Cell signaling	D
LYVE-1	Up	Urine	Receptor binding glycoprotein		D
TFF1	Up	Urine	Regulation of transcription factor	Promoter modeling	D
REG1A	Up	Urine	REG1A protein	Cell signaling	D

NGAL: Neutrophil gelatinase-associated lipocalin; miR: MicroRNA; D: Diagnostic; P: Prognostic.

RNases in blood. They also showcase sufficient levels for quantitative analyses[79]. Living cells consistently release RNA, which is encapsulated inside big lipoprotein complexes, namely exosomes/microvesicles (MVs). RNA that is obtained from dead or dying cells found from blood is connected with apoptotic bodies and protein complexes[80].

Exosomes range from 40-140 nm in diameter and are made of lipoprotein membranous vesicles of endocytic origin. They are formed from the fusion of multivesicular bodies with the plasma membrane. They are released into extracellular spaces thereafter[81]. MVs are much larger than exosomes and have a diameter ranging from 100-1000 nm. They have heterogeneous morphologies, and they originate from the plasma membrane by budding with the extracellular space.

Apoptotic bodies are morphologically MVs that are heterogeneous in shape, spanning 50-500 nm in diameter, and contain organelles. They are released *via* outward protrusion from the plasma membrane

during the late phase of apoptosis[82]. Several studies have demonstrated that tumors specifically secrete exosomes or MVs, which contain specific miRNAs. However, Arroyo *et al*[83] stated that miRNAs in circulation are principally found in the Ago2 ribonucleoprotein complex and not vesicles. This indicates that detected miRNAs are derived mostly from apoptotic and necrotic processes, which occur frequently in tumor cells. These circulating RNAs are found in the plasma or serum of patients and are upregulated when compared to healthy patients.

A significant amount of RNA biomarkers have been shown to be circulating miRNAs and are mostly tissue specific. Some reports found tumor-specific upregulation of various types of small noncoding RNAs (ncRNAs), such as small nuclear RNAs, Piwi-interacting RNAs and long ncRNAs (lncRNAs) like HOTAIR and MALAT1. They act as diagnostic and prognostic markers in the serum or plasma of cancer patients[84,85]. Amidst that, some have reported alterations in gene expression according to disease progression, which discriminates between a chronic inflammatory state and carcinoma (Table 3)[86,87].

Funaki *et al*[88] detected *CEA* mRNA by real-time reverse transcription PCR in the whole blood of PanCa patients. Clarke *et al*[89] also detected *EGFR* mRNA in the serum and detected alpha 1,4-N-acetylglucosaminyltransferase mRNA in the mononuclear cell fraction of peripheral blood from PanCa patients. Kang *et al*[90] further demonstrated that serum-type collagen mRNA acts as good marker of PanCa. It undergoes tumor-specific alternative splicing, which is expected to result in high specificity (Table 3).

In PanCa, the tumor suppressor genes *p53, p16* and *SMAD4* are rendered inactive by the miRNAs, which are produced only in tumor tissues[91]. In pancreatic cell lines and tissue, miR-21 is increased, which drastically lowers the survival rate. In the *KRAS* (G12D) animal model, it has been reported that miR-21 overexpression is the lesion onset point that leads to tumor growth. miR-155 is also overexpressed in PanCa. This aids in the development of tumors. Decreasing the expression of miR-155, *MT1-MMP, KRAS,* and *EGFR* inhibits cell growth. miR-221 is also upregulated in PanCa and promotes distant metastasis as well as unresectable tumors[92]. In approximately 50%-70% of cases of PanCa in humans, the *p53* gene has a point mutation. Hypoxia with malnutrition trigger stress signaling in cells that upregulates *p53*, which increases the expression of numerous genes, including *MIR-107*, *MIR-34a/b/c* and *MIR-34* (Table 3)[15,65,86].

Role of cfRNA as a diagnostic and therapeutic biomarker

According to some reports, miRNA dysregulation has been reported in feces, urine and saliva, which are easy to obtain by non-invasive methods. The levels of miR-143, miR-223 and miR-30 were seen to be higher in the urine of patients with stage I disease. The combination of miR-143 with miR-30 showed very high sensitivity and specificity of 83.3% and 96.2%, respectively. Yang *et al*[93] reported that the levels of miR-21 and miR-155 were much higher in PanCa patient stool when compared with healthy controls. Recently studies revealed that salivary miRNAs were stable because of the protection of exosomes or protein complexes, thus highlighting promising roles as diagnostic markers. *SNHG15* serum expression exerts an average diagnostic value (Table 3)[94].

Liao *et al*[95] found that plasma snoRNA *SNORD33/66/76* might serve as a diagnostic biomarker for non-small cell lung cancer (NSCLC) using comprehensive next generation sequencing analysis. Wang *et al*[96] detected the expression of miRNAs in the plasma of patients with PDAC and identified miRs-21, miR-210, miR-155 and miR-196a. They are upregulated in PanCa tissue and cell lines as candidate biomarkers, and miR-200a/b, miR-18a, miR-221 and miR-196a/b have been found to be upregulated in the serum/plasma in parallel with cancer tissues by comprehensive sequence and microarray analysis (Table 3)[96,97].

Kishikawa *et al*[86] suggested that some miRNAs, such as miR-16, miR-223 and let-7, are highly and constantly expressed in the serum/plasma and are correlated with the number of blood cells. Tang *et al* [98] found higher levels of three lncRNAs, long intergenic non protein-coding RNA 1627 (LINC01627), LINC01628 and ERICH1 antisense RNA 1 (ERICH1-AS1; also known as DLGAP2), in the plasma from patients with NSCLC than in samples from healthy individuals after microarray assay. They proposed a potential diagnostic signature for NSCLC based on these lncRNAs. Anfossi *et al*[99,100] suggested that urine levels of miR-148a and miR-375 have been found to be significantly downregulated and upregulated, respectively, in patients with prostate cancer and enabled patients with prostate cancer to be distinguished from healthy individuals (Table 3).

Higher levels of expression of hsa-miR-106a, hsa-miR-125a-5p, hsa-miR-129-3p, hsa-miR-375, hsa-miR-205, hsa-miR-29b, hsa-miR-21 and hsa-miR-7 in histopathology as well as in cytology samples using real-time reverse transcription PCR act as valuable diagnostic biomarkers in different types of cancer[101]. Also, miR-21, miR-210, miR-155 and miR-196a, which have been upregulated in PanCa tissue and cell lines, have been identified by Wang *et al*[96] as candidate biomarkers. A possible diagnostic biomarker for malignant melanoma is miR-221. Li *et al*[103] found that patients with high serum miR-221 levels had significantly worse 5-year relapse-free survival (12.5% *vs* 45.2%; *P* = 0.008). Increased circulating levels of miR-221 allowed patients with melanoma to be discriminated from healthy individuals (*P* = 0.0001) (Table 3)[103].

Rac1 downregulation leads to the switching of the MKK4-JNK-c-Jun pathway, which silences miR-124. In response to TGF, miR-200a and miR-205 are downregulated in PDAC during epithelial-mesenchymal transformation (EMT), while miR-21 overexpression is identified as the lesion initiator

that drives tumor growth in a *KRAS (*G12D*)* animal model. miR-155, which is elevated in PanCa, aids in the development of tumors.

Karandish *et al*[104] suggested that stress signaling in cells induced by hypoxia and starvation upregulate *p53* and activate the expression of several genes, such as *MIR-107*, *MIR-34a/b/c* and *MIR-34*. Mutation of *p53* mediates transcription of *MIR-130b* and *MIR-155*, which modifies the expression of the corresponding target genes (*ZEB1* and *ZNF652*). This leads to cell proliferation and invasion in several cancers. *ZEB1* has been shown by Wellner *et al*[106] to control the activation of EMT in pancreatic cancer stem cells and to suppress the production of the miR-200 family[104]. In addition, *p53* mutation impairs the maturation of miR-145 and miR-16-1 causing cell proliferation, invasion and migration in PDAC (Table 3).

As a long intergenic non-protein coding RNA, *p53* induced transcript (Linc-pint) is ubiquitously expressed in humans and acts as a direct *p53* transcriptional target. Plasma lncRNA Linc-pint was significantly decreased in patients with PanCa, carcinoma of the ampulla of Vater and cholangiocarcinoma compared with healthy volunteers[108]. Therefore, Linc-pint might be used for identifying the cause of malignant obstructive jaundice and helping to trace the origin of the cancer. Some of these results indicate that low plasma Linc-pint expression could serve as a minimally invasive biomarker for early PanCa detection and that low Linc-pint levels in PanCa tumors could be used for predicting patient prognosis(Table 3)[103].

Prior studies revealed that upregulation of chromatin-interacting lncRNA along with MEG3 inhibited PanCa cell proliferation with the activation of *p53*. Additionally, lncRNA-NUTF2P3-001 contributed to PanCa proliferation and invasion by activating the miR-3923/KRAS signaling pathway. Moreover, LOC389641 promoted the progression of PDAC and increased cell invasion by regulating E-cadherin with the possible involvement of *TNFRSF10A* (Table 3)[109].

The d-signature comprises eight extracellular vesicle long RNAs, including *FGA, KRT19, HIST1H2BK, ITIH2, MARCH2, CLDN1, MAL2* and *TIMP1*, for PDAC detection, and the d-signature could distinguish between CA19-9 negative PDAC cases and healthy controls, thus complementing the use of CA19-9 in PDAC detection. It could also distinguish PDAC from CP with high accuracy. Circ-LDLRAD3 was found to be increased in PanCa tissues as well as in plasma samples. It was markedly related to lymphatic invasion, along with venous invasion and metastasis. Circ-LDLRAD3 has not been reported as an ideal standalone biomarker but may be in combination with CA19-9[110].

The circRNAs *PDE8A* and *IARS* are present in the plasma exosome. They were upregulated along with a concurrent association with prognosis and progression of PanCa. They were also likely to be promising biomarkers in the detection of early-stage PanCa. When compared with the healthy controls, circ-001569 levels were higher. Aberrant levels of *CPA4, PFAA, MUC5AC, GPANCA1, OPNT + TIMP-1* and *C4BPA* were frequently reported in serum samples of PanCa patients. In pancreatic juice samples, the higher regulation of ARG2 signifies its role as a biomarker for PanCa diagnosis. Neutrophil gelatinase-associated lipocalin in urine also provides some clue for early diagnosis of PanCa (Table 3) [111,112].

Role of immunological treatments in PanCa and non-malignant diseases

The lack of immune and targeted molecular therapies is a reason for treatment failure and lower survival rates[113]. The reason behind less efficient immune therapies in PanCa is unclear. Usually, PTs are surrounded by thick desmoplastic stroma making targeted treatments less effective. Other reasons of immune therapy failure are chemokine-initiated barring of T cells followed by comparatively faulty antigenicity when compared to other solid tumors[114]. A model states that the systemic progression of PDAC development initiates from PanIN1 to PanIN3 and finally leads to invasive PDAC. This occurs when high progressive deposition of desmoplastic stroma and alteration of leucocyte infiltration happens[115].

The lower mutation burden in PDAC is partially due to poor antigenicity of the tumor microenvironment (TME). The abundant immunosuppressive TME plays a crucial role in blocking T cell activation, which further leads to lower immune therapy efficiency[116]. A significantly increased level of the *TH17* and *Th1* genes acts as an inflammatory immune marker[117]. A variety of immune biomarkers have been reported in PDAC. Those with sound prognostic capability are *CD3, CD4, CD8* and *CD20*. In contrast, increased expression of *CD163, FOXP3, CD204, CD68* and *CD66b* leads to poorer outcomes[114, 118].

Interestingly, the presence of T cells has no clinical correlation with PDAC. Tumor-infiltrating CD3+ T cells have been identified in isolated PDAC tumors. However, due to the lack of CD3 zeta expression, the capability to activate T cell-associated receptor signaling has been lost and is under functioning [119]. The tumor immune microenvironment and TME of PDAC is peculiar when compared with other solid tumors because immune therapy does not yield significant results in PanCa treatment. PD-L1, CTLA-4 and PD-1 targeting antibodies are part of immune checkpoints, and inhibition of these targets has been successful in other malignancies but have failed in curative treatment of PanCa[120]. Of note, PD-L1 and PD-1 expression is significantly lower in PDAC than in other tumors. PD-L1 has high expression in almost 30%-40% of PanCa cases, which is correlated with CD8+ cells, thereby indicating a poor prognosis[120,121].

Table 4 Cell-free biomarkers involved in distinguishing pancreatic cancer from other pancreatic benign diseases

Biomarkers	CP	AIP	PanIN	IPMN	MCN
PENK DNA	Yes	No	Yes	Yes	Yes
CUX2	Yes	No	No	No	No
REGIA	Yes	No	No	No	No
TFP12	Yes	No	Yes	Yes	Yes
FOXE1	Yes	No	Yes	Yes	Yes
P16	Yes	No	Yes	Yes	Yes
NPTX2	Yes	No	Yes	Yes	Yes
PENK	Yes	No	Yes	Yes	Yes
CYCLIN D2	Yes	No	Yes	Yes	Yes
CDO1	No	Yes	No	No	No
HAS-mIR-23a	No	No	No	Yes	No
HAS-mIR-23b	No	No	No	Yes	No
HAS-mIR-210	Yes	No	No	No	No
Let-7c	Yes	No	No	No	No
S100A9	Yes	No	No	No	No
BNCI	No	No	Yes	No	No
ADAMTS1	No	No	No	No	No
BNC1	No	No	No	No	No
9-Sep	No	No	No	No	No
GPC1	No	No	No	No	No
PFAA	No	No	No	No	No
OPNT + TIMP-1	No	No	No	No	No
CPA4	No	No	No	No	No
MUC5 AC	No	No	No	No	No
C4BPA	No	No	No	No	No

AIP: Autoimmune pancreatitis; CP: Chronic pancreatitis; IPMN: Intrapapillary mucinous neoplasia; MCN: Mucinous cystic neoplasm; PanCa: Pancreatic cancer; PanIN: Pancreatic intraepithelial neoplasia.

CONCLUSION

Currently, a variety of specimens have emerged as possible sources for liquid biopsy analysis for diagnostic applications. Apart from plasma or serum, urine and sputum have also produced promising disease diagnostic results[44,122]. Liquid biopsies are ideally non-invasive or minimally invasive for sample collection, minimizing patient stress and discomfort. Non-invasive approaches can significantly aid clinical research and utility. Even the recurrence and metastasis of the disease can be potentially monitored based on the cfDNA methods (Table 4).

Therefore, in this review we highlighted the current development in tools for PanCa early diagnosis and prognosis. We demonstrated that useful data can be obtained based on DNA methylation signatures and mutations. The heterogeneity in the pool of cfDNA isolated from a suitable source, such as plasma, contains cfDNA molecules obtained from various origins, and various outcomes, such as DNA methylation or gene mutations, could be obtained from such material. The process of properly understanding the disease source and cfDNA origin has been facilitated significantly by advancements in technologies, such as single cell-based techniques[40]. These advancements can further facilitate liquid biopsy research associated with PanCa biomarker discovery. This also showcases that aberrant DNA methylation signatures harness a significant capability to guide PanCa disease management[32, 51]. Approximately 300 clinical trials are currently ongoing that target the use of cfDNA biomarkers in the diagnosis of malignancies. Limitations include cfDNA isolation and sample processing and understanding the biology of ctDNA based on its release from tumors.

The outcome of the secretome can also support PanCa diagnosis significantly. Premalignant lesions such as PanIN are still facing detection challenges because no PanIN-specific protein has been clinically approved. The alterations in cell free nucleic acids does not always manifest into protein changes that can be clinically validated and utilized. However, the secretome in PanCa and secretome biomarkers specific to premalignant lesions are yet to be identified for clinical use but are likely to be highly useful in the future. Validation of biomarkers based on cfDNA alterations and subsequent protein expression can substantially help in the early diagnosis of PanCa and other pancreatic-associated diseases[51,123].

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FOOTNOTES

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