Molecular prognostic markers in pancreatic cancer: A review

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ABSTRACT
Pancreatic cancer is one of the most aggressive human malignancies. Its incidence in the United States has tripled in the past 50 years and the tumor is a frequent cause of cancer death in both men and women. In this review we discuss the common molecular alterations and their value as markers of prognosis for pancreatic ductal adenocarcinomas. We describe cytogenetic abnormalities, alterations of oncogenes and tumor suppressor genes, of cell receptors and growth factors, and of factors modulating tumor/stromal interaction playing a role in pancreatic ductal adenocarcinoma. The impact of these changes on cell proliferation, tumor progression and tumor apoptosis is discussed. Finally, a review of gene expression alterations (mRNA microarray and microRNA) and of quantitative proteomic analysis of pancreatic cancer is also presented. Some of the molecules described herein have been shown to represent potential biomarkers of tumor prognosis, and/or of tumor response to therapy. These biomarkers may soon be implemented in the clinical practice to improve the management of patients with pancreatic ductal adenocarcinomas.

KEYWORDS: pancreas, cancer, molecular markers, prognosis

INTRODUCTION
Histologically the pancreatic parenchyma is divided into two components, the exocrine portion comprised of ducts and acini, and the endocrine component made of hormone secreting cells arranged in islets (islets of Langerhans). Pancreatic carcinoma (PCA) usually arises in the exocrine component of the gland, and almost the totality of these tumors originates from the epithelium lining the pancreatic duct. The ductal cells acquire over time a series of sequential molecular alterations transforming them first into dysplastic cells, pancreatic intraepithelial neoplasia (PanIN) of increasing grade, and finally to carcinoma. Eventually, carcinoma leads to metastatic foci (Fig. 1). Acinar cell tumors comprise less than 1% of the pancreatic malignant tumors [1]. Tumors arising from the islets of Langherans are called islet cell tumors, and comprise 1 to 2% of all pancreatic cancers [2].

Pancreatic ductal adenocarcinoma (PDCA) is more common in African-American males, and in patients with either diabetes mellitus, or hereditary chronic pancreatitis. Most of these tumors occur after the age of 60, and involve the head of the pancreas [2]. The incidence of PDCA has increased three fold in the last 50 years, especially in women [3]. This increase is probably related to changes in diet, exposure to cigarette smoking, and chemical carcinogens. The problem with this type of cancer is that it grows rapidly...
and without specific symptoms in early development, so that when it is diagnosed it is usually widespread and unresectable [2].

PCA is the fourth leading cause of cancer death in the United States, and the eighth most common cause of cancer mortality worldwide. Its incidence and mortality rates are almost identical. The 5-year survival rate is about 1 to 2%, and the median survival time after diagnosis is 4-6 months. The American Cancer Society estimates that there will be 43,140 new cases of PCA in 2010 in the USA, with 36,800 estimated PCA deaths in the same year [3]. These observations attest the ineffectiveness of current treatment modalities for this form of human cancer, and our limited knowledge of the pathogenesis of PDCA. This paper deals with some of the molecular alterations identified, so far, in PDCA and discusses their value as predictors of tumor prognosis and/or of tumor response to therapy.

**Cytogenetic abnormalities**

Cytogenetic analysis of PDCA have identified alterations in the form of gene rearrangement or losses in chromosomes 1p, 3p, 6q, 8p, 12p, 16q. Loss of chromosomes 17 and 18, which carry the p53 and DCC genes, are also common [4]. Adsay et al. using fluorescent in situ hybridization on a group of 10 PDCAAs identified the frequent loss of chromosome 20, alterations of chromosome 8, and the amplification of c-myc oncogene [5]. Recently, Salek et al. have reported that allelic

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**Fig. 1.** Progression model for pancreatic ductal adenocarcinoma. Normal duct epithelium acquires a sequential series of molecular alterations, to transform into precursor lesions (PanINs), and then to progress to carcinoma and lymph node metastasis (left to right). The overexpression of HER2/neu and KRAS mutations occur early; inactivation of p16, STAT3 activation, and MUC4 expression at an intermediate stage; and the inactivation of p53, SMAD4, and BRCA2 as well as cathepsin B and MIX1 expression, overexpression PCNA, EGFR, uPA, and expression BIM1 and CD44 occur relatively late.
loss of 9p and 18q do not predict prognosis in PDCA [6].

**DNA ploidy and cell proliferation**

Studies using image cytometry and/or flow cytometry have shown that a non diploid or aneuploid DNA content is usually associated with advanced tumor stage and shorter survival [7]. Tsavaris et al. evaluating PDCAs from 226 patients, reported that patients with DNA ploidy score > 3.6 had 5.0 times higher probability of death in comparison with patients with DNA ploidy score < 2.2 [8].

Pancreatic tumor cells also express high proliferating cell nuclear antigen (PCNA) as compared to adjacent non-neoplastic tissue, a finding that may be useful in supporting the diagnosis of malignancy when only a small biopsy specimen is available for pathologic interpretation [9]. PCNA overexpression in PDCA correlates with the presence of lymph node metastasis [9]. Similarly, the Ki-67 proliferative index is higher in PDCA as compared to non-neoplastic pancreas (3.73 +/- 3.58 vs. 37.03 +/- 10.05) [9], and it was found to correlate with liver metastases and short survival [10].

**Oncogenes and tumor suppressor genes**

Mutations and/or overexpression of p53 have been detected in 45-75% of human pancreatic carcinomas, but are not associated with prognosis in some studies [10, 11]. However, others have reported that patients with PDCAs carrying a mutated p53 have shorter survival after radiation and/or chemotherapy, as compared to patients with wild type p53 [12]. Recently it has been shown that p53 overexpression and chromosome 17 numerical imbalances may be present in the same significant percent of pancreatic cancers [13].

Wild type p53 when sensing DNA damage, has the capability of inducing p21/WAF1, a cyclin-dependent kinase inhibitor able to arrest cell proliferation. Cell cycle arrest is important to assess the extent of DNA damage, and to activate gene repair enzymes. If the DNA damage cannot be repaired p53 induces BAX, an apoptotic protein that will eliminate the mutated cell. A mutated p53 is unable to provide these protective functions [14]. The association between cigarette smoking and risk of developing PDCA is known [15]. However, while an association was found between cigarette smoking and the presence of K-ras mutations in PDCA, this risk factor was not associated with the presence of p53 mutations [16].

P16, is a tumor suppressor gene, encoding for a protein that acts as a cyclin dependent kinase (CDK) 4/6 inhibitor [17]. Loss of p16 has been found in a significant percentage of pancreatic cancers [18] and p16 alterations have been correlated with reduced survival in some studies [19, 20].

BRCA2 is another tumor suppressor gene encoding a nuclear protein with multiple regulatory functions on gene transcription, chromatin remodeling, cell growth, DNA damage repair, and chromosomal stability [21]. While somatic mutations of BRCA2 are rare and mutations in BRCA1 appear to have a limited role in pancreatic cancer, germline mutations of BRCA2 have been associated with the development of PDCA [22]. However, Skoulidis et al. using a murine model of familial pancreatic cancer have shown that BRCA2 LOH may not be essential for pancreatic ductal carcinogenesis in mutation carriers, and that a significant proportion of tumors in these patients may retain a functional BRCA2 allele. Therefore, poly-ADP-ribose polymerase (PARP1) inhibitors, which selectively target BRCA2-deficient cancer cells, should be used after tumor LOH has been confirmed [23].

Recent evidence suggests a role of β-catenin pathway in PDCA. The β-catenin gene (CTNNB1) was reported to be mutated in poorly differentiated PDCAs, resulting in activation of the WNT/β-catenin pathway and poor prognosis [24, 25].

Mutations of the K-ras oncogene have been identified in approximately 80% of PDCAs, however, its significance as a prognostic marker for PDCA is debatable [25, 26]. For example, Iacobuzio-Donahue et al. found K-ras mutations in 95% of their 59 patients, but they only reported DPC4 loss to be predictive of metastasis [26]. Conversely, Chen et al. in their study of 91 unresectable pancreatic cancer patients, found mutated K-ras in 33% of them. In these patients
Epidermal growth factor (EGF) and platelet derived growth factor (PDGF) are produced by pancreatic cancer cells [38], and overexpression of epidermal growth factor receptor (EGFR) in PDCA is a marker of metastatic disease [39]. The use of monoclonal antibodies against TGF-alpha and heparin-binding EGF-like growth factor frequently secreted by pancreatic tumor cell lines, greatly inhibited cancer cell growth, both in vitro and in tumor-bearing animals [40].

Weak HER2 expression has been reported in 43 to 69% of pancreatic cancers [41], and the use of anti-HER2 monoclonal antibody (mAb) has shown to have a synergistic therapeutic effect when combined with an anti-EGFR mAb on pancreatic carcinomas with low HER2 expression [42].

Factors involved in tumor/stromal interaction

The poor prognosis of pancreatic cancer is dependent on its invasive and metastatic capabilities. PDCA is especially prone to invasion of the surrounding tissues and to metastasis. The expression of CD44, a transmembrane glycoprotein involved in cell-to-cell and cell-to-matrix interactions, is increased in pancreatic cancer and it seems responsible for gemcitabine resistance and poor prognosis [43]. Variant isoforms of CD44, and especially variant CD44V6, are known to increase the metastatic potential of pancreatic carcinoma cells, and may represent a new therapeutic target for PDCA [44]. CD44 variants 6 and 2, preferentially expressed in pancreatic cancer cells, correlate with decreased overall survival [45, 46]. Another study has shown the involvement of CD44v10 in mediating fibronectin adhesion in vitro, and in counteracting metastases in vivo [47].

Lysosomal cathepsins B, D and L may promote carcinogenesis and tumor progression [48]. In particular, cathepsin B catalyzes the degradation of laminin, with consequent degradation of the basement membrane and facilitation of tumor invasion and metastasis [48]. Recently, hedgehog gene has been reported to signal through cathepsin B to influence pancreatic cancer cell invasiveness [49]. It has also been shown that human mesenchymal stem cells (MSC) migrate from the mutated K-ras correlated with stage, presence of metastasis, and it was predictive of poor prognosis [27]. This finding may have clinical applications as the K-ras status may be determined from plasma DNA of unresectable pancreatic cancer patients [27].

Growth factors and cell receptors

We and others have observed the overexpression of insulin-like growth factor-1 (IGF-1) receptor and the activation of tyrosine kinase Src in human pancreatic ductal adenocarcinoma [28, 29]. Src is a cytoplasmic membrane - associated protein tyrosine kinase involved in the regulation of cell growth and differentiation, and cell adhesion [30]. Targeted inhibition of Src kinase has been shown to slow-down the growth of orthotopic PDCA [31], and to impair its drug resistance [32]. It seems that the activation of Src may induce the IGF1-dependent proliferation of pancreatic cancer cells by increasing the number of IGF1 receptors per tumor cell [33].

Constitutive activation of STAT3 is another molecular alteration implicated in pancreatic carcinogenesis. For example, benzyl isothiocyanate (BITC), a compound found in cruciferous vegetables, has been shown to induce apoptosis of pancreatic cancer cells by inhibiting the STAT3 pathway [34]. The activated JAK2, STAT3, and STAT5 can also be inhibited, in vivo, by using the triterpenoid compound cucurbitacin. Such treatment also inhibits other components of the JAK/STAT pathway (p21, cyclin A, cyclin B, and Bcl-XL), inducing apoptosis and inhibiting proliferation of pancreatic cancer cells [35].

In addition to the IGF1/IGF1-R system mentioned above, human pancreatic cancer cells express a variety of other growth factor receptors and of their ligands that may provide selective growth advantage. For example, 60% of sporadic PDCA harbor mutations of transforming growth factor-β (TGF-β)/SMAD4, and mutations of SMAD4 were shown to be associated with metastasis and to predict poor prognosis [11, 36]. TGF-beta expression in PDCA influences the cell cycle and suppresses PTEN through NF-kappaB activation, enhancing cell motility and invasion in a SMAD4-independent manner [36, 37].
In young patients it was found to correlate with shorter DFS and to have a trend toward lower OS. High SGLT1 expression in primary tumors correlated with high Bcl-2 expression but not with p53 expression [62].

Greten et al., using a mice model (EL-TGFalpha-hGH transgenic mice crossbred to p53-deficient mice) observed that transcription factors STAT3 and NF-kappaB upregulated Bcl-xL expression in the premalignant lesions of PDCA. The authors suggested that the combined inhibition of STAT3 and NF-kappaB might represent a novel strategy for tumor prevention and therapy [63]. Survivin expression was also studied in PDCA and it was found to be associated with venous or perineural invasion. The authors concluded that survivin is associated with metastasis and it may be a marker of poor prognosis as well as a marker able to predict response to chemotherapy [64].

Most recently, Song et al. studied the expression in PDCA of B-cell-specific Moloney murine leukemia virus insertion site 1 (BMI1), a member of the polycomb group of transcriptional repressors. BMI1 expression was found to be markedly up-regulated in pancreatic cancer cell lines as well as in surgically resected cancer specimens. The authors described the positive correlation between BMI1 expression and the presence of lymph node metastases. BMI1 was also found to negatively correlate with patient survival rates, suggesting a role for BMI1 in the progression of PDCA [65].

Finally, we have investigated the role of the human cellular apoptosis susceptibility protein (hCAS) in human pancreatic cancer. hCAS is a Ran- binding protein playing a role as mitotic spindle checkpoint regulator and as nuclear export factor. hCAS expression is frequently altered in metastatic cancers [66]. We found that hCAS protein expression was decreased in serum starved MiaPaCa-2 cells, as compared to fetal bovine serum stimulated cells. When compared to Src transfected MiaPaCa-2, Src knockdown-MiaPaCa-2 cells demonstrated lower proliferation, increased apoptosis, as well as inhibition of malignant transformation. Pancreatic ductal carcinomas (PCA) were hCAS strongly positive. Normal pancreatic ducts (ND) and PanIN (all grades) were hCAS weakly positive. While the hCAS antibody decorated only the cytosolic compartments of ND
MIX1 was identified and validated by real time PCR and immunolabelling. Upregulation of MIX1 may occur during cancer progression to refine c-myc oncogenic signal to protect neoplastic cells from induced c-myc apoptosis [84]. The redundancy observed in published studies for many of the highly expressed genes identified in pancreatic cancers support the belief that these genes play an important role in the pathogenesis of pancreatic cancer.

A recent comprehensive genetic analysis of 24 PDCA using microarray containing probes for -10^6 single-nucleotide polymorphism found that PDCA have 63 genetic alterations and that most of these are point mutations. The genes implicated are components of 12 molecular pathways regulating cell cycle, apoptosis, DNA damage control, tumor invasion and metastasis [85].

MicroRNAs
MicroRNAs (miRNAs) are small non-coding RNA molecules (19-24 nucleotides) that possess regulatory functions in gene expression. They regulate their targets by direct mRNA cleavage or translational inhibition [86, 87]. Aberrant expression of miRNAs contribute to carcinogenesis by promoting the expression of proto-oncogenes or by inhibiting tumor suppressor genes [88]. Recent miRNA expression profiling studies of pancreatic cancer have identified a large number of aberrantly expressed miRNAs that may represent a unique miRNA signature. miR-221, miR-222, miR-155, and miR-21 have consistently been reported in several studies as being dysregulated in pancreatic cancer [89-90]. The high expression of miR-196a-2 was found to predict poor survival [87, 89]. However, these are preliminary data and further validation studies are required. In the future, the characterization of these miRNAs as prognostic and/or diagnostic markers in pancreatic cancer may provide avenues for their activation or silencing as a novel therapeutic strategy.

Cyclooxygenase-2 Exyclooxygenase-2 expression in human pancreatic cancer
Recent studies have underlined the potential role of cyclooxygenase-2 in human pancreatic carcinogenesis. Cyclooxygenases COX-1 and COX-2 are enzymes necessary for the conversion of arachidonic acid to prostaglandin H2, a precursor of other prostaglandins, prostacyclin, and tromboxanes [68]. COX-2 expression is induced by growth factors, cytokines and oncogenes, and that COX-2, but not COX-1, is overexpressed in a variety of epithelial neoplasms including pancreatic carcinoma [69-72]. It is becoming evident that specific COX-2 inhibitors can prevent carcinogenesis and induce apoptosis of tumor cells [73, 74]. Lipton et al. have recently reported that the combination of gemcitabine, irinotecan, and celecoxib is an effective therapy for inoperable pancreatic cancer [75].

Gene expression profiling
Gene expression technologies, including representational difference analysis, serial analyses of gene expression, and microarrays, have discovered numerous differentially expressed genes in pancreatic cancer as compared with normal pancreas and chronic pancreatitis, in order to find novel markers to detect pancreatic cancer at an earlier stage and to develop targets for drug development [76, 77]. Among the differentially expressed genes identified, MUC 4, fascin, mesothelin, and topoisomerase IIα, have been repeatedly reported as being highly expressed in pancreatic cancer [78-82]. MUC 4 has been reported to be expressed at high levels in pancreatic intraepithelial neoplasia, as well as, in invasive pancreatic cancer indicating possible association with premalignant lesions [82]. In addition, MUC 4 reportedly is significantly correlated with poor survival [83]. In advanced pancreatic cancer associated with locally destructive behavior, an increase expression of MIX1 was identified and validated by real time PCR and immunolabelling. Upregulation of MIX1 may occur during cancer progression to refine c-myc oncogenic signal to protect neoplastic cells from induced c-myc apoptosis [84]. The redundancy observed in published studies for many of the highly expressed genes identified in pancreatic cancers support the belief that these genes play an important role in the pathogenesis of pancreatic cancer.

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Proteomics
Quantitative proteomics technology uses a comparative approach for protein profiling to identify deregulated proteins associated with disease [91, 92]. Recent proteomics studies in
pancreatic cancer utilizing either pancreatic juice, pancreatic cancer tissue (whole cancer tissue and microdissected cancer cells), serum or plasma, and pancreatic cell lines have identified potential candidate protein biomarkers [91-93]. S100A6, fascin, and cathepsin D are proteins found to be overexpressed in pancreatic cancer tissue and further validated and confirmed by immunohistochemistry [92-94]. Additional proteins have been detected, however, many of them frequently occur in the setting of pancreatitis [92]. Proteomics profiles of chemo-resistant pancreatic cancer cell lines have identified a few novel proteins, including E-FABP, coflin, and 14-3-3 sigma, that may be involved in the development in chemotherapy resistance [93-95]. Unfortunately, these studies use a variety of methods and sample sources which in trying to compare the different investigations become problematic. Patient populations are poorly characterized with inadequate numbers to enable statistic analyses and the histology/classification of pancreatic tumors studied is rarely documented. The reproducibility is low and validation studies on independent populations are needed [94-95]. Hopefully, the limitations present are transient and proteomic technology with better resolution, sensitivity, quantification, and reproducibility will be created to provide more consistent platforms for validating candidate protein biomarkers.

DISCUSSION

In summary, we described numerous molecular alterations reported in PDCA (Table 1). Recent studies have tried to decipher which alterations are significant to patient prognosis and overall survival. It is becoming evident that the co-expression of Bcl-2 and Bax may represent a marker of good prognosis. On the other hand, B-catenin mutations, SMAD4 mutations, and increased expression of CD44 and MUC4 are associated with poor prognosis. Similarly, aneuploid DNA content, p53 mutation, high Ki-67 index, and high expression of miR-196a-2 reportedly correlate with a shorter patient survival.

Several other molecular alterations may predict the tumor biology. For example, aneuploid DNA content not only demonstrates a significant correlation with shorter survival but has been associated with a higher tumor stage. Cathepsin B and uPA are expressed in invasive and metastatic PDCA. Additionally, overexpression of EGFR, variant CD44v6, and survivin correlate with metastatic disease. Lymph node metastasis was reportedly associated with PCNA over-expression. All of these clinically relevant molecular abnormalities are summarized in Table 2.

It is possible that monitoring of selected molecular alterations may allow gauging the aggressiveness of a patient’s PDCA and provide additional therapy when needed. In selected cases, the presence of specific molecular alterations may direct clinicians to the use of a “personalized” treatment for PDCA. One of such examples is given by a pancreatic cancer containing activated STAT3. These tumors have been shown to respond to Cucurbitacin, a drug inhibiting activated STAT3 and other components of the JAK/STAT pathway, causing arrest of tumor cell proliferation and inducing their apoptosis [28].

Interestingly, the presence of certain cellular alterations also affects tumor response to therapy. Down regulation of Tissue Transglutaminase (TG) in pancreatic cancer enhances the therapeutic efficacy of gemcitabine [48]. Moreover, other molecular alterations may induce tumor resistance to particular chemotherapeutic drugs. This information would provide clinicians the option of selecting an alternative therapy and/or to save patients from side effects of ineffective drugs. Similarly, increased CD44 expression appears to impart gemcitabine resistance, and inhibition of Src kinase has been shown to impair PDCA drug resistance [25]. Clinically, detection of CD44 overexpression may indicate the use of a different drug modality or may infer the use of two drug modalities which could have a synergistic effect against pancreatic cancer cells (Table 2). Surprisingly, the significance of K-ras mutations, identified in the majority of PDCA, as prognostic markers, is controversial.

Through the use of various techniques, a number of molecular abnormalities have been identified within PDCA. Selected molecular alterations have enhanced our knowledge of tumor biology and aided in determining patient prognosis, and in assigning better therapeutic modalities. Additional
Table 1. Molecular abnormalities in pancreatic ductal adenocarcinoma.

<table>
<thead>
<tr>
<th>Cytogenetics</th>
<th>Losses in chromosomes 1p, 3p, 6q, 8p, 12p, 16q</th>
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<tr>
<td></td>
<td>Loss of chromosomes 17 and 18</td>
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<td></td>
<td>Amplification c-myc oncogene</td>
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<td>DNA Ploidy</td>
<td>Non diploid or aneuploid</td>
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<tr>
<td>Oncogenes</td>
<td>K-ras mutation</td>
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<tr>
<td>Tumor suppressors</td>
<td>p53 mutation and/or overexpression</td>
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<tr>
<td>Tyrosine kinases</td>
<td>Src overexpression or activation</td>
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<tr>
<td>Growth factors</td>
<td>TGF alpha and beta, IGF-1, beta chain PDGF</td>
</tr>
<tr>
<td>Cell receptors</td>
<td>Overexpressed: EGFR, c-erb-B2 proto-oncogene receptor, and IGF-1 receptor</td>
</tr>
<tr>
<td>Factors involved in tumor/stromal reaction</td>
<td>CD44 expression (variants 6 and 2), cathepsin B, uPA, TG</td>
</tr>
<tr>
<td>Apoptotic alterations</td>
<td>Bax expression, Bcl-xL expression</td>
</tr>
<tr>
<td>Enzymes</td>
<td>COX-2 expression</td>
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<tr>
<td>Enhanced gene expression</td>
<td>MUC 4, fascin, mesothelin, topoisomerase IIa</td>
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<tr>
<td>miRNA expression</td>
<td>miR-221, miR-222, miR-155, miR-21, miR-196a-2</td>
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<tr>
<td>Overexpressed proteins</td>
<td>S100A6, fascin, cathepsin D</td>
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Table 2. Molecular abnormalities and their associated clinical significance.

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>Molecular alteration</th>
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<tr>
<td>Good prognosis</td>
<td>Co-expression of Bcl-2 and Bax</td>
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<tr>
<td>Poor prognosis</td>
<td>B-catenin mutations, SMAD4 mutations, CD44 overexpression, MUC4 expression</td>
</tr>
<tr>
<td>Short survival</td>
<td>Non diploid/Aneuploid DNA content, Ki-67 high proliferative rate, p53 mutations, miR-196a-2 high expression</td>
</tr>
<tr>
<td>Advanced tumor stage</td>
<td>Non diploid/Aneuploid DNA content</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td>EGFR overexpression, Variant CD44v6 expression, Survivin expression</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>PCNA overexpression</td>
</tr>
<tr>
<td>Liver metastasis</td>
<td>Ki-67 high proliferative rate</td>
</tr>
<tr>
<td>Therapeutic characteristics</td>
<td>CD44 expression associated with gemcitabine resistance, Down regulation of TG enhances efficacy of gemcitabine, Src kinase impairs PDCA drug resistance</td>
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</tbody>
</table>
studies are required to not only find additional molecular abnormalities which could be therapeutic targets, but to further confirm the significance of present molecular alterations and their association to patient prognosis and survival.

In the future, the availability of specific gene signatures predictive of prognosis and therapy response may become available, allowing a “personalized” treatment for PDCA.

**ABBREVIATIONS**

TGF (transforming growth factor), IGF-1 (insulin-like growth factor - 1), PDGF (platelet derived growth factor), EGFR (epidermal growth factor receptor), uPA (urokinase plasminogen activator), TG (tissue transglutaminase), COX-2 (cyclooxygenase-2)

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**CONFLICT OF INTEREST**

The authors have no conflict of interest to disclose.

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