

REVIEW ARTICLE

MicroRNAs as Indicators and Predictors of Pancreatic Adenocarcinoma

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ABSTRACT

Chronic pancreatitis is a chronic inflammatory disorder, triggered by various factors. Several factors increase the risk of the development of pancreatic adenocarcinoma, including age, smoking, and chronic alcohol consumption. Predictors for cancer development are currently not available in the stage of chronic pancreatitis; and technical procedures such as endoscopic ultrasound or MRI scanning techniques are often hampered by a low specificity of cancer prediction. Thus, pancreatic cancer is often diagnosed at advanced stages, and chemotherapy does not improve the outcome dramatically. A reliable test in blood, tumor or bile would help to detect the tumors at earlier stage. Most interestingly, a new predictive parameter has recently evolved since MicroRNAs have been associated with cancer development and progression, and recent scientific evidence suggests that MicroRNAs can be regarded as “tumor markers” since several molecules are expressed at high levels in the tumors themselves throughout different patient groups. MicroRNAs expression profiles even seems to classify human gastrointestinal cancer better than MicroRNAs –protein expression profiles. Among the various molecules, miR-21, miR-34a, miR-198, miR-155 and miR-217 have been shown to be highly specific as biomarkers differentiating between chronic pancreatitis and pancreatic ductal adenocarcinoma. Also, MicroRNAs-196a and -196b have been described as potential biomarkers for the early detection of familial pancreatic cancer. Pilot studies now aim to develop a diagnostic test for pancreatic ductal adenocarcinoma based on differential expression of select MicroRNAs in plasma and bile, not only to predict cancer development but also to understand molecular basis of cancer progression.

INTRODUCTION

Prognosis and treatment options of pancreatic cancer are still limited because pancreatic cancer is often diagnosed in an advanced tumor stage. According to the American cancer society, only 9% of all pancreatic cancers are diagnosed in a localized tumor stage, whereas 56% of patients had distant metastasis at diagnosis stage [1]. Therefore, treatment is often restricted to palliative regimes. Regardless of the underlying tumor stage, overall 5 years survival is about 6% [1]. In general tumor stage of pancreatic cancer is categorized into surgically resectable, locally advanced (LA) and metastatic pancreatic cancer. R0 resection is considered to be the only potentially curative approach in pancreatic cancer. However, due to non-detectable distant metastasis and a high rate of local recurrences, the 5 years survival rate after R0 resection is about 20% [1]. Although pancreatic cancer is still lethal in most cases, new, encouraging diagnostic new molecular tool have been established in the last years, which will be presented and discussed in the following.

Current Diagnostic Approaches in Pancreatic Cancer

Painless jaundice is a characteristic sign of pancreatic cancer, but it is almost always associated with an advanced tumor stage. Characteristic early symptoms are missing in pancreatic cancer, some patients have complained about chronic abdominal pain but did not get accurate diagnosis. Interestingly, onset of diabetes mellitus can be an early sign of pancreatic cancer. A meta-analysis of 88 studies [2] found a strong association between a recently diagnosed diabetes mellitus and pancreatic cancer. These data suggest that patients with new-onset DM should be screened for pancreatic cancer. Until quite recently, routine preoperative biliary drainage was performed in patient undergoing surgery. However, van der Gaag *et al.* [3] compared in a multicenter randomized trial routine preoperative biliary drainage versus surgery alone. This landmarks study clearly showed that rates of serious complications were significantly higher in the biliary-drainage group than in the early surgery group [74% vs. 39% (0.54; 95% CI, 0.41 to 0.71)]. Therefore, preoperative biliary drainage in patients undergoing surgery is currently only recommended in patients with cholangitis. Furthermore, there is no need to perform a biopsy for histological proof, unless patients are undergoing a chemotherapeutic treatment or imaging findings are not clear. The reason for this reserved attitude towards histological proof is the fear of tumor cell dissemination along the needle track. However, this fear appears to be

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unfounded in practice. A previously published study [4] analysed the long-term outcomes of patients with resected pancreatic cancer and preoperative Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA). The authors found that preoperative EUS-FNA was not associated with increased risk of mortality and concluded that EUS-FNA is a safe approach to assess the dignity of suspicious pancreatic lesions. Furthermore, EUS FNA testing new molecules could become a safe standard, and new parameters will be discussed in the following.

MicroRNAs: New Molecular Tool in Pancreatic Adenocarcinoma

An new and interesting predictive parameter has recently evolved since MicroRNAs have been associated with cancer development and progression, underlining a potential for predictive markers [5, 6, 7].

MicroRNAs (MicroRNAs) are non-protein coding ~22 nucleotide RNAs that induce translational repression and/or degradation of their MicroRNAs targets. MicroRNAs are small noncoding RNAs that are cleaved from 70-100 nucleotide hairpins pre-MicroRNAs precursors in the cytoplasm by RNAase III Dicer into their mature form of 22 nt. Single stranded MicroRNAs bind messenger RNA of potentially hundreds of genes at the 3 untranslated region with perfect or near perfect complementarity, resulting in degradation or inhibition of the target messenger RNA. In humans, aberrant expression of MicroRNAs contributed to carcinogenesis by promoting the expression of proto oncogenes or by inhibition of tumor suppressor genes. MicroRNAs, in complex with AGO protein, uses seed sequences near the 5' end to base pair with a target MicroRNAs, thereby inducing de-adenylation and decay or translational regulation [8]. A growing body of evidence not only suggests that bacterial and viral infection of mammalian and plant cells can modulate MicroRNAs expression, but recent data also suggest a key role of these molecules in cancer development and progression [8].

MicroRNAs expression profiles seem to classify human gastrointestinal cancer better than MicroRNAs-protein expression profiles, as demonstrated recently in a new approach [9]. The recent work [9] used a bead-based flow cytometric MicroRNAs expression profiling method to present a systematic expression analysis of 217 mammalian MicroRNAs from 334 samples, including multiple human cancers. The group observed a general down-regulation of MicroRNAs in tumours compared with normal tissues. Furthermore, they were able to successfully classify poorly differentiated tumours using MicroRNAs expression profiles, whereas messenger RNA profiles were highly inaccurate when applied to the same samples. The report suggested that one of the key roles of MicroRNAs expression is the post-transcriptional silencing of targeted genes, the enhancement of proteins being translated, or the prevention of apoptosis by binding to promoter units involved in cell cycle regulation, and these findings highlight the potential of MicroRNAs profiling in cancer diagnosis.

MicroRNA Expression in Chronic Pancreatitis and Pancreatic Cancer

In particular, diagnosis of pancreatic cancer in patients with chronic pancreatitis is challenging, because transition of a chronic inflammatory induced tumor mass into a malignant process is blurred and difficult to distinguish by morphological imaging and histopathological procedures [10]. The typical biology of pancreatic ductal adenocarcinoma is one of aggressive local invasion, early metastasis, and resistance to chemotherapy and radiation. Known genetic mutations include TP53, KRAS, CDKN2A, and SMAD 4, but these factors do not account for aggressive behavior.

In a comprehensive work published by Bloomston *et al.* [11], global expression patterns in patients with chronic pancreatitis and pancreatic ductal adenocarcinoma were identified, and this work described a more than 95% accuracy of seven MicroRNAs molecules: miR-99, miR-100, miR100-1/2, miR-125a, miR-125b, miR-199a1 and miR199-a2. Furthermore, expression of miR196-a-2 had a significant impact on survival. Some of the molecules that may have an interesting role for survival are known as hypoxia related MicroRNAs. Data were confirmed in further studies of the same group in later years [12, 13, 14].

This work also described the difference of the MicroRNAs content between the two different groups. Six different MicroRNAs were differentially expressed at more than 2-fold difference: miR-21, miR-148a/b, miR-155, miR-181a/b/c/d, miR-221 and miR-275 [11]. Most notably, miR-21 and miR-155 were uniquely overexpressed in pancreatic cancer vs normal pancreas and chronic pancreatitis [11]. miR-21 has been suggested to play an important role in preventing apoptosis, thus functioning as a proto oncogene. The most highly expressed MicroRNAs in pancreatic cancer was miR-21, when comparing cancer with chronic pancreatitis. MicroRNAs 221 is important in thyroid cancer, and suggested to play a role in angiogenesis. Far fewer molecules were downregulated in this study, most notably miR-375 normally found in pancreatic islets.

As illustrated in table 1, numerous studies have been performed regarding the functional and biological role of MicroRNAs in pancreatic adenocarcinoma, and results are shown in **Table 1**.

In most recent works, Le Large *et al.* have pointed out that circulation MicroRNAs may be used as diagnostic biomarkers for pancreatic cancer, and the circulating MicroRNAs molecules can be determined in blood as diagnostic biomarkers [15, 16]. Interestingly, label-free nanoplasmonic-based short noncoding RNA Sensing at attomolar concentrations seem to allow for quantitative and highly specific assay of MicroRNA-10b in biological fluids and circulating exosomes [17]. Also, MicroRNAs in stool samples have been described as potential screening biomarkers for pancreatic ductal adenocarcinoma cancer [18]. Circulating MicroRNAs in Pancreatic Juice as Candidate Biomarkers of Pancreatic Cancer [19]. A MicroRNA meta-signature for pancreatic ductal adenocarcinoma [20].

Table 1. Functional and biological role of MICRO RNAs in pancreatic cancer.

Name	Functional Role	Biological Role	References
miR-10b	Increased expression of MicroRNAs-10b, -155, and -106b in plasma appears highly accurate in diagnosing PDAC		[24]
miR-21	miR-21, miR-34a and miR-217 could be used as tumor makers to distinguish PDAC and its precursors from a benign lesions	Expression level of miR-21 is significantly higher in pancreatic ductal adenocarcinomas compared to healthy tissues and tissues of chronic pancreatitis; increased expression of miR-21 was significantly associated with shorter disease free survival and overall survival	[21]
	High levels of miR-21 were associated with a poor response to gemcitabine		[10]
	MiR-21, miR-155 and miR-216 in stool have the potential of becoming biomarkers for screening PDAC		[18]
		High miR-21 tumoural expression independently prognostic for poor overall-survival	[20]
		MicroRNA-21 is overexpressed in pancreatic cancer and potential predictor of survival	[13]
miR-31		High miR-31 tumoural expression independently prognostic for poor overall-survival	[20]
miR-34a	miR-21, miR-34a and miR-217 could be used as tumor makers to distinguish PDAC and its precursors from a benign lesions	Expression level of miR-34a is significantly higher in pancreatic ductal adenocarcinomas compared to healthy tissues and tissues of chronic pancreatitis	[21]
miR-106b	Increased expression of MicroRNAs-10b, -155 and -106b in plasma appears highly accurate in diagnosing PDAC		[24]
miR-155	MiR-21, miR-155 and miR-216 in stool have the potential of becoming biomarkers for screening PDAC		[18]
	Increased expression of MicroRNAs-10b, -155 and -106b in plasma appears highly accurate in diagnosing PDAC		[24]
miR-196a	The combination of miR-196a and -196b may be a promising biomarker test for the screening of IAR for FPC		[22]
miR-196b	The combination of miR-196a and -196b may be a promising biomarker test for the screening of IAR for FPC		[22]
miR-198	miR-21, miR-34a and miR-217 could be used as tumor makers to distinguish PDAC and its precursors from a benign lesions	Expression level of miR-198 is significantly higher in pancreatic ductal adenocarcinomas compared to healthy tissues and tissues of chronic pancreatitis	[21]
		Increased expression of miR-198 was significantly associated with shorter disease free survival and overall survival	[12]
miR-199a1/a2	Potential biomarker for pancreatic adenocarcinoma		[11]
miR-205	Elevated levels of circulating miR-205, miR-210, miR-492 and miR-1247 in pancreatic juice are promising candidate biomarkers of disease and poor prognosis in patients with PDAC		[19]
miR-210	Elevated levels of circulating miR-205, miR-210, miR-492 and miR-1247 in pancreatic juice are promising candidate biomarkers of disease and poor prognosis in patients with PDAC		[19]
miR-216	miR-21, miR-155 and miR-216 in stool have the potential of becoming biomarkers for screening PDAC		[18]
miR-217	miR-21, miR-34a, miR-198 and miR-217 could be used as tumor makers to distinguish PDAC and its precursors from a benign lesions	Expression level of miR-217 is significantly higher in pancreatic ductal adenocarcinomas compared to healthy tissues and tissues of chronic pancreatitis	[21]
miR-375		Low miR-375 tumoural expression independently prognostic for poor overall-survival	[20]
miR-486-5p	miR-486-5p and miR-938 were able to discriminate PDAC patients from healthy controls and whose with chronic pancreatitis. The diagnostic ability of miR-486-5p for identifying PDAC from healthy controls was comparable to that of CA 19-9		[16]
miR-492	Elevated levels of circulating miR-205, miR-210, miR-492 and miR-1247 in pancreatic juice are promising candidate biomarkers of disease and poor prognosis in patients with PDAC		[19]

miR-938	miR-486-5p and miR-938 were able to discriminate PDAC patients from healthy controls and those with chronic pancreatitis.	[16]
miR-1247	Elevated levels of circulating miR-205, miR-210, miR-492 and miR-1247 in pancreatic juice are promising candidate biomarkers of disease and poor prognosis in patients with PDAC	[19]

Recent studies using Roc analysis (ROC: receiver operating characteristics) indicated that MicroRNAs could be extracted and detected from pancreatic juice and stool efficiently and reproducibly, and that MiR-21, miR-155 and miR-216 in stool have the potential of becoming biomarkers for screening PDAC [18]. The data using a combination of MicroRNAs were obtained through processing the original expression data of miR-21, miR-155 and miR-216 by logistic regression analysis. Receiver operating characteristic curve (ROC) analysis was performed to assess the diagnosing value of stool MicroRNAs in PDAC patients; and these data indicated that a combination of miR-21 and miR-155 had best sensitivity of 93.33% while the combination of miR-21, miR-155 and miR-216 would be best for detecting and screening PDAC with area under the curve (AUC) of 0.8667 (95% CI: 0.77-0.96) and a better balance of sensitivity and specificity (83.3% vs. 83.3%). Obviously, miR-21, miR-155 and miR-216 in stool seemed to have the potential of becoming biomarkers for screening PDAC.

Recent reports further support the view that among the various molecules, especially miR-21, miR-34a, miR-198 and miR-217 can be regarded as highly specific as diagnostic biomarkers for chronic pancreatitis and pancreatic ductal adenocarcinoma [21]. In this work, expression of the microRNAs miR-198, miR-217, miR-21 and miR-34a was determined in normal tissue, tissue of chronic pancreatitis and pancreatic adenocarcinoma (PDAC). While the expression of miR-198 increased from normal tissue to CP and PDAC, the expression of miR-217 decreased in a opposite fashion. The other two only showed specific expression in tumor tissue. Thus, MiR-21 and miR-34a seem to be excellent tissue biomarkers differentiating chronic pancreatitis from PDAC.

Also, MicroRNA-196a and 196b were confirmed as potential biomarkers for the early detection of familial pancreatic cancer [22]. Interestingly, a pilot study to develop a diagnostic test for pancreatic ductal adenocarcinoma based on differential expression of select MicroRNAs in plasma and bile, which is currently under further investigation [23, 24].

CONCLUSION

MicroRNAs (MicroRNAs) are non-protein coding ~22 nucleotide RNAs that induce translational repression and/or degradation of their mRNA targets. In surgical specimens obtained from pancreatic tumor tissue as well as normal or chronically inflamed pancreatic tissue, miR-21, miR-34a, miR-155, miR-196-a/b, and miR-198 have been shown to be highly specific biomarkers differentiating between chronic pancreatitis and pancreatic ductal adenocarcinoma. Pilot

studies now aim to develop a diagnostic test for pancreatic ductal adenocarcinoma based on differential expression of select MicroRNAs in plasma and bile. Furthermore, tumor specimens obtained through endoscopically guided fine needle aspiration could present a new tool to test the presence of such MicroRNAs molecules, allowing for new cancer markers, not only to predict cancer presence but also to understand molecular basis of cancer progression.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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