

MicroRNAs in gastrointestinal cancer: a novel biomarker and its clinical application

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ABSTRACT

Gastrointestinal (GI) cancers remain one of the most common malignancies and are the major cause of cancer deaths worldwide. Significant advancements have improved our understanding of the pathogenesis and pathology of GI cancers, but high mortality rates, an unfavorable prognosis, and lack of clinical predictive biomarkers provide an impetus to investigate novel diagnostic/prognostic markers and therapeutic targets for GI cancers. MicroRNAs (miRNAs) are short (19-24 nucleotides), non-coding RNA molecules that regulate gene expression at the post-transcriptional level, thus playing an important role in modulating various biological processes. This includes developmental processes, proliferation, apoptosis, metabolism and differentiation, all involved in initiation and progression of various human cancers. Aberrant miRNA expression profiles have been observed in various cancer types at different stages, suggesting their potential as diagnostic and prognostic biomarkers. Due to their tumor- and tissue-specific expression profiles, stability, and the availability of robust clinical assays for their detection in serum as well as in formalin-fixed tissue samples, miRNAs have emerged as attractive candidates for diagnostic and prognostic applications. This review summarizes recent research supporting the utility of miRNAs as novel diagnostic/prognostic tools and therapeutic targets, thus potentially illuminating future treatment strategies for GI cancers.

Key words: Biomarker, gastrointestinal cancer, microRNA, therapeutic target

Introduction

Gastrointestinal (GI) cancers represent malignant tumors of the GI tract and accessory organs of digestion including esophagus, stomach, liver, biliary tract, pancreas, small intestine, large intestine and rectum. GI cancers are collectively the major cause of cancer-related morbidity and mortality worldwide.^[1] Current multimodal treatment strategies including surgery, radiotherapy, and/or chemotherapy have marginally improved curative expectations and quality of life of patients; however, the effectiveness of these new tools depends largely on the stage in which tumors are detected. Previous investigators have tried to identify more specific and sensitive novel biomarkers and therapeutic targets for better diagnosis and management of lethal GI cancers.

MicroRNAs (miRNAs) are short, non-coding RNA molecules of approximately 19-24 nucleotides involved in post-transcriptional regulation of gene expression. miRNAs bind to the 3'-untranslated region of mRNA, leading to either translational repression or

mRNA degradation initiated by miRNA-guided rapid deadenylation.^[2] It has been estimated that 60% of human protein coding genes are subject to regulation by miRNAs.^[3] They act as master regulators for many important biological processes including ontogeny, cell proliferation, apoptosis, migration, differentiation, metabolism, stress, viral infection, cancer initiation and progression and drug resistance.^[4-7] In addition, several miRNAs may also be useful for diagnostic, prognostic and therapeutic applications in GI cancers.^[8-12]

Numerous investigations on screening for altered expression of miRNAs in various types of cancer have been conducted during the past decade, with more and more functional validations in recent years. Although the majority of such studies have so far focused on miRNA profiling to identify specific miRNA species and determining their role in the biology of GI cancers, another great potential for miRNA profiling lies in their

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use as biomarkers, either in diagnosis or in prediction/monitoring of therapeutic responses. This review focuses on the most recent advances in studies on some extensively investigated miRNAs in GI cancers, particularly with regard to their potential as novel biomarkers or therapeutic targets.

Esophageal Cancer

The incidence and mortality of esophageal cancer (EC) are high, ranking eighth and sixth respectively, all types of cancer, affecting more males than females.^[13] Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the two main EC subtypes. Due to the potential characteristics of invasion and metastasis in esophageal carcinoma cells, the overall 5-year survival rate is poor despite advanced treatment.^[13,14] Recent discoveries have shed new light on the involvement of miRNA in EC.^[15]

miRNAs as novel diagnostic and prognostic biomarkers in EC

Guo *et al.*^[16] found aberrant expression of 46 miRNAs in EC tissues, of which 7 miRNAs may be used as biomarkers to distinguish malignant EC lesions from adjacent normal tissue. Moreover, miR-335, miR-181d, miR-25, miR-7 and miR-495 are associated with the pathological type of EC (fungating vs. medullary). miR-25 and miR-130b are associated with the degree of differentiation of EC and miR-103/107 expression level negatively correlated with survival rates miR-25 and miR-130b may also be used for early diagnosis as well as gene therapy targets for EC. Feber *et al.*^[17] found that miR-203 and miR-205 were down-regulated compared with normal epithelium in EC, while miR-21 was overexpressed in the two types of EC. miR-200c, miR-194 and miR-192 were up-regulated in EAC. Mathe *et al.*^[18] have demonstrated that the overexpression of miR-21 in non-cancerous tissue of ESCC and down-regulation of miR-375 in cancerous tissue of EAC with Barrett's esophagus (BE) were markedly associated with a worse prognosis. miR-196a was highly expressed in EAC, BE, benign and malignant junctions and highly malignant tissue and thus be used as a biomarker for screening EC.^[18-20] Among others, the overexpression of miR-129 was identified as a significant and independent prognostic factor in surgically treated ESCC patients.^[21] The expression level of miR-1322 was higher in ESCC tissue, making it possible to distinguish ESCC from healthy samples.^[22] Expressions of miR-31 and miR-142-3p correlated with histological differentiation, with high miR-142-3p expression being associated with poor prognosis. Therefore, this may be a potential independent prognostic ESCC factor.^[23] Furthermore, expression profiles of miRNAs have been found to be altered in progressive stages of EC neoplastic development, with expression levels of miR-31 and miR-31* being frequently down-regulated in EAC.^[24]

Other significant miRNAs with dysregulated expression are miR-16-2 and miR-30e, which were associated with a shorter overall and disease-free survival in all EC patients.^[25]

Several studies have demonstrated that miRNAs are consistently detectable in the circulation. The expression level of miR-21 was up-regulated and miR-375 was down-regulated in plasma of ESCC patients compared with healthy controls; patients with high plasma levels of miR-21 had greater vascular invasion and showed high correlation with recurrence.^[26] A panel of 7 serum miRNAs (miR-10a, miR-22, miR-100, miR-148b, miR-223, miR-133a and miR-127-3p) was up-regulated in ESCC and could clearly distinguish stage I/II ESCC patients from controls.^[27] Supporting the role of miRNAs in the circulation, Zhang *et al.*^[28] have found that miR-31 levels were significantly higher than controls in 523 serum ESCC samples. In addition, patients with higher levels of serum miR-31 had a poorer prognosis for relapse-free survival. miR-1322 was significantly highly expressed in ESCC serum and could be used to distinguish ESCC from healthy patients.^[22] Thus, circulating miRNAs may be used as potential biomarkers, not only for diagnostic, but also for prognostic and predictive markers in EC.

Clinical application of microRNAs in EC

Hummel *et al.*^[29] examined the impact of chemotherapy on miRNA expression in EC cells and found that 13 miRNAs were deregulated following treatment with cisplatin or 5-fluorouracil (5FU). miR-141 was most highly expressed in the cisplatin-resistant ESCC cell lines, the target of miR-141 is YAP1, which is an apoptosis-inducing gene in DNA-damaging agents.^[30] miR-296 and miR-27a are overexpressed in EC, and knockdown of miR-296 and miR-27a was found to be capable of increasing sensitivity to both P-glycoprotein-related and P-glycoprotein-non-related drugs, in turn promoting ADR-induced apoptosis in EC cells.^[31,32] Overexpression of miR-200c significantly correlated with response to chemotherapy, with this effect being associated with the Akt pathway.^[33] miR-148a up-regulation significantly increased the sensitivity to chemotherapy in the majority of cells.^[34] miR-200b/200c/429 were up-regulated in endometrial cancer and EC, and their overexpression correlated with resistance to cisplatin treatment.^[35]

Gastric Cancer

Gastric cancer (GC) is the second leading cause of cancer-related death worldwide. Approximately one million new GC cases per year were estimated to have occurred in 2010.^[36] *Helicobacter pylori* infection, Epstein-Barr virus infection, gastrin levels, germline mutations, dietary factors, and other chronic gastric conditions are all factors felt to be involved in GC development. GC is often diagnosed at an advanced

stage, accompanied by extensive invasion and lymphatic metastasis.^[37] Thus, it is important to increase the sensitivity and specificity of diagnostic markers and/or to establish methods for GC treatment and prevention of GC.^[38-40]

miRNAs as novel diagnostic and prognostic biomarkers in GC

Novel methods, such as circulating miRNA profiling, have been suggested to be useful tools for the non-invasive diagnosis of GC. Three serum miRNAs (miRs-221/744/376c) were found to distinguish GC patients from healthy controls with 82.4% sensitivity and 58.8% specificity.^[41] Moreover, miR-221 and miR-376c demonstrated significantly positive correlations with poor GC differentiation.^[41] In a validation experiment, plasma levels of miR-451 and miR-486 were higher in patients with GC compared with healthy controls, with high area under the curve (AUC) values (0.96 and 0.92).^[42] A genome-wide miRNA profile identified high serum levels of miR-378 in patients with GC, and validation yielded a high AUC (0.86).^[43] Quantitative real-time polymerase chain reaction analysis identified 5 serum miRNAs (miR-1, miR-20a, miR-27a, miR-34, miR-423-5p) to be biomarkers for GC, and their levels correlated with tumor stage.^[44] Plasma concentrations of miRNAs miR-17-5p, miR-21, miR-106a and miR-106b were higher, whereas let-7a was lower in GC patients. AUC as high as 0.879 for the miR-106a/let-7a ratio assay was achieved.^[45] High levels of miR-17 and miR-106a in peripheral blood of GC patients confirmed in another study, in which the AUC value for the combined miR-17/miR-106a assay was 0.741.^[46] These findings suggest that miRNAs are useful biomarkers for early GC diagnosis.

miRNAs have recently been used to predict the outcome of patients with GC. For example, a seven-miRNA signature (miR-10b, miR-21, miR-223, miR-338, let-7a, miR-30a-5p and miR-126) was shown to be closely associated with relapse-free and overall survival (OS) among patients with GC.^[47] High expression of miR-20b, miR-150^[48] and miR-93^[49] or down-regulation of miR-451^[50] or miR-218^[51] was also associated with poor survival, whereas there was a correlation between miR-27a and lymph node metastasis.^[48] In addition, Ueda *et al.*^[52] recently reported that miR-125b, miR-199a and miR-100 represent a progression-related signature, whereas the low expression of let-7g and high expression of miR-214 were associated with shorter OS independent of the depth of invasion, lymph node metastasis and stage.

Circulating miRNAs have been suggested to be useful prognostic markers of GC. High expression of circulating miR-17-5p/20a was an independent poor prognostic factor.^[53] Low-level expression of let-7a/let-7g/let-7f was associated with a poor prognosis.^[54,55]

miR-181b and miR-182 were also found to be novel poor prognosticators.^[56] Low expression of miR-125a-3p was associated with enhanced malignant potential, such as tumor size, lymph node, and liver metastasis, and poor prognosis, and this study suggested that miR-125a-3p is a potent prognostic marker in GC.^[57] Furthermore, miR-409-3p was found to be frequently down-regulated in patients with GC, and its expression was associated with distant metastasis.^[58]

Clinical application of miRNAs in GC

Some miRNAs have been shown to impact chemotherapy sensitivity if their levels were artificially up-regulated, others if they were down-regulated. For instance, up-regulation of miR-21 or miR-106a increased cisplatin resistance of GC cells,^[59] and Deng *et al.*^[60] showed that the up-regulation of miR-195 or miR-378 led to enhanced 5-azacytidine resistance in normal gastric cells. Up-regulation of miR-449 or miR-508-5p was demonstrated to positively impact sensitivity toward cisplatin (miR-449) or vincristine or doxorubicin (miR-508-5p).^[61,62] Interestingly, in accordance with these findings concerning the modulation of the sensitivity toward chemotherapeutic drugs via miRNAs, Bandres *et al.*^[50] reported that the up-regulation of miR-451 led to increased sensitivity of cancer cells toward radiotherapy by down-regulating macrophage migration inhibitory factor MIF. Only one research group reported the effect of miRNA down-regulation on chemotherapy resistance; Zhao *et al.*^[63] found that increased doxorubicin sensitivity in GC cells was connected with down-regulation of miR-27a.

Colorectal Cancer

Colorectal cancer (CRC) is the 3rd most common cancer and the 3rd leading cause of cancer-related death in the world, with an estimated incidence of one million new cases and a mortality of > 500,000 deaths annually.^[13] The pathogenesis of CRC typically follows a protracted stepwise progression from benign adenoma to malignant adenocarcinoma and distant metastasis, rendering screening and early diagnosis as preferred options to ease the disease burden.^[64] This also highlights the need for the development of novel screening tools and diagnostic biomarkers.

miRNAs as novel diagnostic and prognostic biomarkers in CRC

Ng *et al.*^[65] were the first to report that circulating levels of miRNAs differed in the blood plasma in CRC cases and the controls. It was found that miR-92 was expressed at higher levels in the plasma from CRC cases and could distinguish cases from healthy control patients with 70% specificity and 89% sensitivity. Another study of 120 cases and 29 controls validated these findings, showing that levels of miR-92 can discriminate between CRC cases and controls with 65% sensitivity and 82% specificity.^[66]

A similar study found that levels of miR-141 were elevated in metastatic CRC and its expression was associated with a poor prognosis, suggesting that this miRNA may be used in conjunction with carcinoembryonic antigen to detect CRC with distant metastases.^[67]

Measuring miRNAs in stool offers another non-invasive approach to detect CRC. One small study of 29 CRC cases and 8 healthy controls found that stool from CRC cases expressed higher levels of miR-21 and miR-106a.^[68] A larger study of 197 cases and 134 healthy controls investigated miRNA expression patterns of colonocytes isolated from feces and was able to demonstrate that miRNA expression patterns could distinguish cases from controls with 74% sensitivity and 79% specificity.^[69] A similar strategy found that miRNA methylation patterns from DNA isolated from stool may be promising as a screening tool for CRC.^[70] The hypermethylation pattern of miR-34b/c in stool samples could distinguish CRC cases from controls with 75% sensitivity and 84% specificity. Further tests are warranted to determine whether miRNA expression or methylation patterns in stool can be utilized, either alone or in combination with a fecal occult blood test, as an effective screening strategy for CRC.

The elevated expression of miR-21 has a robust and reproducible association with the CRC prognosis. Schetter *et al.*^[71] first reported that elevated miR-21 expression in tumors was associated with a worse survival prognosis and therapeutic outcome. The association of elevated miR-21 expression and worse survival outcomes in CRC has been validated in at least three additional studies. These include the studies of 156 Japanese CRC patients,^[72] 46 CRC patients from the Czech Republic,^[73] and 130 tumor node metastasis stage II colon cancer patients from Denmark.^[74] Additional studies have identified miRNA expression patterns that are associated with either prognosis or therapeutic outcome. Expression levels of miR-106b,^[75] miR-320,^[76] miR-498,^[76] miR-125b,^[77] miR-145,^[78] miR-185,^[79] miR-133b,^[79] miR-215^[80] and miR-17^[81] have each been reported to be associated with prognosis or therapeutic outcome. An elevated expression of Dicer, an important gene encoding an RNA nuclease involved in miRNA processing, is associated with poor prognosis in CRC.^[82] Further validation of these associations is warranted and may reveal additional prognostic classifiers.

Clinical application of miRNAs in CRC

Schetter *et al.*^[71] have shown that miR-21 expression is associated with therapeutic outcome with 5FU-based therapies. This association, in combination with the known oncogenic role for miR-21, suggests that increased miR-21 expression is, in part, responsible for resistance to 5FU. Elevated miR-21 induces resistance to 5FU in colon cancer cell lines by down-regulating DNA repair protein MutS homolog 2.^[83] Exposure of colon cancer

cells to 5FU leads to increased miR-21 expression, and this may be a response to genotoxic stress to help cells overcome the effects of 5FU.^[84] Additional *in vitro* data support the roles for altered expression of miR-140,^[85] miR-215,^[86] miR-224^[87] and miR-20a^[88] in developing chemoresistance. Further studies are warranted to determine whether expression of these miRNAs can predict response to chemotherapy and if those miRNAs can be used as therapeutic targets themselves. MiRNA replacement involves reintroducing synthetic miRNA mimics or expression vectors that will produce the miRNA of interest. This has shown promise in preclinical murine models where the reintroduction of miR-145 and miR-33a had an antitumor effect in a model of colon cancer.^[89]

Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC), the most common primary liver cancer, is the 5th most frequent cancer and the third cause of cancer-related mortality worldwide.^[90] The incidence of this disease is > 600,000 cases annually.^[91,92] HCC usually develops as a consequence of underlying liver disease and is often associated with cirrhosis.^[93] Hepatitis B virus (HBV) and hepatitis C virus (HCV) viral infections, the major risk factors for HCC development, lead to liver cirrhosis and account for 75% of HCC cases.^[94,95] miRNAs have been widely reported to be involved in HCC development and may be new targets for HCC therapy.^[96-98]

miRNAs as novel diagnostic and prognostic biomarkers in HCC

Many miRNAs are dysregulated in HCC; thus, it is to be expected that circulating miRNA levels are also affected by HCC progression. The high stability of miRNAs in circulation makes them excellent biomarkers, especially for early detection.^[99] It is interesting that circulating miR-21,^[100,101] miR-222^[101] and miR-223,^[102] were up-regulated in serum/plasma of HCC patients associated with HBV or HCV. Circulating miR-21 levels were significantly higher in HCC patients than in those with chronic hepatitis and healthy controls. A receiver-operating characteristic analysis of miR-21 yielded an AUC of 0.773 when differentiating HCC from chronic hepatitis, and an AUC of 0.953 when differentiating HCC from healthy controls. Both sets of values were superior to alpha-fetoprotein (AFP) as an HCC biomarker.^[102] At the same time, the serum levels of miR-1, miR-25, miR-92a, miR-206, miR-375 and let-7f were also significantly elevated.^[103]

Serum miR-15b and miR-130b levels were also up-regulated in HCC.^[104] MiR-130b had the largest AUC (0.913), with a sensitivity of 87.7% and specificity of 81.4%, and miR-15b had the highest sensitivity of miRNAs examined (98.3%), although its specificity was very low (15.3%). The high sensitivity of circulating

miR-15b and miR-130b as biomarkers holds promise for patients with early-stage HCC, who may have low AFP levels despite the presence of disease. Similarly, serum miR-16 was found to be a more sensitive biomarker than serum AFP and Des- γ -carboxyprothrombin (DCP).^[105] The combination of miR-16, AFP, AFP-L3%, and DCP yielded the optimal combination of sensitivity (92.4%) and specificity (78.5%) for HCC overall and when the analysis was restricted to patients with tumors smaller than 3 cm.^[106] In addition, a recent meta-analysis of 8 studies showed the diagnostic value of miRNAs as follows: Pooled sensitivity 0.87 (0.72-0.98), pooled specificity 0.90 (0.76-1.00), pooled positive likelihood ratio 8.7 (3.52-97.45), pooled negative likelihood ratio 0.13 (0.02-0.31), and pooled diagnostic odds ratio 86.69 (19.06-2646.00).^[107]

Although sensitivity and stability of miRNAs as biomarkers are suitable for a clinical setting, appropriate controls must be used in a research setting because HCC is often accompanied by viral hepatitis, cirrhosis, or other underlying liver conditions.^[108] When assessing the specificity of an miRNA for detecting HCC, it is critical to ensure that patients and controls are matched, not only by age and sex, but also by etiology and severity of the underlying liver disease.

Clinical application of miRNAs in HCC

Recently, miravirsin, a locked nucleic acid-modified DNA phosphorothioate antisense oligonucleotide against miR-122, became the first miRNA-targeting drug to receive permission for clinical use.^[109] It was developed to target HCV, as the stability and propagation of this virus are dependent on a functional interaction between the HCV genome and miR-122.^[110,111] Miravirsin resulted in a dose-dependent reduction in HCV levels, without major adverse events and with no escape mutations in miR-122 binding sites of the HCV genome.^[109] The success of miravirsin is promising, not only as a novel anti-HCV drug, but also as the first trial of miRNA-targeting therapy. In addition to miravirsin, a clinical trial of MRX34 (miRNA Therapeutics, Austin, TX, USA) as a mimic of miR-34 is underway. MRX34 is a liposome-formulated mimic of the tumor suppressor, miR-34. Further study of MRX34 is being conducted by miRNA Therapeutics, which initiated a Phase I study in May 2013 to examine effects of MRX34 on unresectable primary liver cancer or advanced or metastatic cancer with liver involvement. If these oligonucleotide therapies are successful, then therapeutic options based on the numerous miRNAs deregulated during hepatocarcinogenesis appear to be promising.^[112]

Cholangiocarcinoma

Cholangiocarcinoma (CCA) is one of the most common malignancies derived from bile duct epithelial cells.^[113] Due to slow growth, late metastasis, and lack of effective

screening methods, CCA is rarely diagnosed during early stages of the disease when surgical procedures are most effective.^[114] Histopathological analyses suggest that the presence of primary sclerosing cholangitis, chronic biliary irritation, or choledochal cysts may predispose individuals to CCA.^[115] More recently, studies have identified a role for miRNAs in the development of CCA by altering different cholangiocyte features such as cell cycle, proliferation, migration and apoptosis.^[116-118]

miRNAs as novel diagnostic and prognostic biomarkers in CCA

The study by Meng *et al.*^[119] was the first to hint at the potential of miRNAs as biomarkers. It was found that miR-21 and miR-200b expression levels were predictors of gemcitabine resistance.

By sequencing and comparing the small RNA libraries of two CCA cell lines to one of a normal biliary epithelial line, Kawahigashi *et al.*^[120] identified and confirmed miR-22, miR-125a, miR-127, miR-199a/a0, miR-214, miR-376a and miR-424 as specifically expressed in normal biliary epithelial cells, but down-regulated in CCA cell lines, suggesting their use as biomarkers for diagnosis. Chen *et al.*^[121] took the approach of using miRNA sensor constructs to compare spatiotemporal activity of six miRNAs (miR-21, miR-200a, miR-200b, miR-146a, miR-155 and miR-221) in primary tissue blocks of CCA and normal control tissue grown from three patients using adeno-associated viral infections. They were unable to identify a definitive pattern between activity of each miRNA and presence of CCA over the entire time frame but when focusing on 24 h post-infection the miRNA profiles, displayed significant differences between CCA and control as well as between patients, suggesting these miRNAs play an active role in CCA. Karakatsanis *et al.*^[122] evaluated the expression levels of several miRNA species in intrahepatic cholangiocellular carcinoma and their prognostic significance. Although miR-21, miR-31, and miR-223 were found to be up-regulated and miR-122, miR-145, miR-200c, miR-221, and miR-222 to be down-regulated, the group was unable to find any correlation with clinical or pathological features. McNally *et al.*^[123] tried to investigate the predictive role of miRNAs on survival in resected CCAs and found 2 of 43 miRNA species evaluated to have the best correlation with survival. Up-regulation of miR-151-3p (41.5 months vs. 12.3 months) correlated better than down-regulation of miR-126 (21.9 months vs. 15.1 months). However, concomitant dysregulation of both showed the best overall correlation with survival (58.7 months vs. 15.1 months).

In a novel approach using bile, Li *et al.*^[117] were able to demonstrate the presence of miRNAs in extracellular vesicles in bile and analyzed the miRNA expression of 74 different species that could be reliably amplified. By using a multivariate organization of the combinatorial

alterations, they were able to establish a miRNA-based panel of five different miRNA species that were able to distinguish CCA from primary sclerosing cholangitis or other biliary obstructions. This may offer an original way to make an early diagnosis of CCA.

Clinical application of miRNAs in CCA

Meng *et al.*^[119] provided the first evidence for the involvement of miRNAs, as well as its first therapeutic use in CCA. When they compared the miRNA expression patterns between malignant and non-malignant human cholangiocytes, miR-21, miR-141 and miR-200b were highly overexpressed in malignant cells and inhibition of miR-21 and miR-200b sensitized cells to gemcitabine. Treatment of xenografts with gemcitabine changed several miRNA expression levels and modulated phosphatase and tensin homolog-dependent PI3 kinase signaling. Okamoto *et al.*^[124] sought to investigate the role of miRNAs in chemoresistance and compared miRNA profiles of two CCA cell lines distinguished by gemcitabine resistance. They identified miR-29B, miR-205 and miR-221 whose ectopic overexpression could restore gemcitabine sensitivity in the resistant cell line. They showed that knockdown of two predicted targets, PIK3R1 (miR-29b and miR-221 target) and MMP-2 (miR-29b target only), via small interfering RNA conferred the same level of gemcitabine to the resistant cell line. They showed that miR-125a-5p was up-regulated in the resistant cell line and that inhibition of miR-125a-5p inhibited cell proliferation in that cell line independent of chemoresistance.

Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy with a poor prognosis due to advanced stage disease at initial diagnosis, frequent recurrence, and the absence of treatment strategies that specifically and effectively target these tumors.^[125] Only 15% of PDAC patients are candidates for surgical resection at the time of diagnosis.^[126] Chemotherapy is considered to be the main treatment option for unresectable cases, while chemoradiotherapy may improve the survival and quality of life.^[127,128] However, PDAC is still extremely resistant to the currently available regimens. Exploring miRNAs as therapeutic targets and biomarkers for the diagnosis and prognosis of PDAC is of interest.^[129-131]

miRNAs as novel diagnostic and prognostic biomarkers in pancreatic cancer

Kong *et al.*^[132] found that three serum miRNAs, including miR-196a, were differentially expressed in PDAC compared with control groups. Another investigation by Wang *et al.* showed that the expression levels of four miRNAs in plasma (miR-21, miR-210, miR-155 and miR-196a) were significantly higher in patients with PDAC.^[133] Li *et al.*^[134]

measured 735 circulating miRNAs in PDAC cases, and controls sera and miR-1290 were found to show the best diagnostic performance. Kawaguchi *et al.*^[135] found that plasma miR-221 concentrations were significantly higher in PDAC patients than those with benign pancreatic tumors and controls. In recent studies, miRNAs were also found to be useful as diagnostic markers for precursor lesions of PDAC. Caponi *et al.*^[136] found that miR-21 and miR-155 were up-regulated in invasive intraductal papillary mucinous neoplasms (IPMNs) compared with non-invasive IPMNs. Further multivariate analyses showed that high miR-21 expression emerged as an independent prognostic biomarker in invasive IPMNs with a poor survival. Lubezky *et al.*^[137] also found miRNAs were useful in identifying IPMN with high risk for malignant transformation. The expression levels of 15 miRNAs, including miR-217, miR-216a, miR-21 and miR-155, were significantly different between two IPMN subgroups (low/moderate-grade dysplastic IPMNs vs. high-grade dysplastic IPMN) and invasive cancer with IPMN. Pancreatic cysts are a group of lesions with heterogeneous malignant potential. Farrell *et al.*^[138] showed miR-21 and miR-221 in pancreatic cyst fluid was associated with invasive cancer.

With regard to survival, strong miR-21 expression was predictive of poorer outcomes compared with absent or faint/focal miR-21 expression in patients with node-negative PDAC.^[139] Jamieson *et al.*^[140] found that high expression of miR-21 and reduced expression of miR-34a were significantly associated with a poor OS in global miRNA microarray expression profiling. Frampton *et al.*^[141] found that a high level of a combination of miR-21, miR-23a and miR-27a was associated with shorter survival times after surgical resection. While strong expression of miR-21 predicted limited survival in PDAC patients, high expression of miR-200c, a member of the miR-200 family, is a good prognostic sign.^[142,143] Elevated levels of miR-155, miR-203, miR-210 and miR-222 expression in PDCA were significantly associated with an increased risk of death compared to patients with reduced expression of these miRNAs.^[144] A subgroup of six miRNAs (miR-452, miR-105, miR-127, miR-518a-2, miR-187 and miR-30a-3p) was found to identify long-term survivors with node-positive disease from those dying within 24 months.^[145] In addition, increased expression levels of miR-155, miR-203, miR-210 and miR-222 were found to be significantly associated with poorer survival.^[144,146] In some recent studies, reduced expressions of miR-218^[147] and miR-130b^[148] in PDAC tissues were found to correlate with a poor prognosis.

Clinical application of miRNAs in pancreatic cancer

Some prognostic miRNAs also play a role in the efficacy of anticancer therapy, thus presenting themselves as new therapeutic possibilities. For instance, PDAC cells

expressing elevated levels of miR-21 are chemoresistant to gemcitabine and reduce the efficiency of apoptosis induction.^[148,149] miRNAs can be targeted. For example, inhibition of mir-21 by its antagonist led to the cessation of tumor growth and induction of apoptosis *in vitro* and *in vivo* (animal experimental model).^[150] Another therapeutic option comes from a possibility-of-function recovery of miR-34a, a potent pro-apoptotic component involved in p53-mediated apoptosis. As shown by Ji *et al.*,^[151] restoration of miR-34a may substitute the function of inactivated TP53 gene. It has been shown that miR-10a promotes the metastatic behavior of the pancreatic cancer (PC) and that its expression is regulated by retinoids.^[152] The use of retinoic acid receptor antagonists inhibit miR-10a expression and stop metastasis of PDAC cells.^[152] In contrast, miR-146a suppresses invasion of PC cells. However, its expression is lowered in PC compared to normal pancreatic tissue. Finally, use of non-toxic natural compounds which increase expression of miR-146a is also considered to be a promising approach to block both invasion and metastases.^[153]

Perspective

Many miRNAs can be used in the diagnosis of cancer, in determining the patient prognosis or as therapeutic targets. We must develop more stringent protocols for collecting and analyzing samples (to avoid variations in sample processing or histologic characterization) and to validate each finding in large independent cohorts. This approach will lead to the selection of the best candidate miRNAs for further study, the development of highly reproducible results, and reduction in inter-study discrepancies. New miRNAs are continuously being discovered, and profiling technologies are rapidly changing. Thus, creating a standardization process for integrating data will be challenging. Development of effective *in vivo* delivery systems is also required if miRNAs are to be used as therapeutics.

Although many additional studies must be performed, miRNAs appear to have many useful clinical applications for patients with GI cancers and other GI disorders. The GI community should look forward to these studies with great anticipation.

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There are no conflicts of interest.

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