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MicroRNAs (miRNAs) as Biomarker(s) for Prognosis and Diagnosis of Gastrointestinal (GI) Cancers

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Abstract

Gastrointestinal (GI) cancers remain one of the most common malignancies and are the second common cause of cancer deaths worldwide. The limited effectiveness of therapy for patients with advanced stage and recurrent disease is a reflection of an incomplete understanding of the molecular basis of GI carcinogenesis. Major advancements have improved our understanding of pathology and pathogenesis of GI cancers, but high mortality rates, unfavorable prognosis and lack of clinical predictive biomarkers provide an impetus to investigate new sensitive and specific diagnostic and prognostic markers for GI cancers. MicroRNAs (miRNAs) are short (19–24 nucleotides) noncoding RNA molecules that regulate gene expression at the posttranscriptional level thus playing an important role in modulating various biological processes including, but not limited, to developmental processes, proliferation, apoptosis, metabolism, differentiation, epithelial-mechenchymal transition and are involved in the initiation and progression of various human cancers. Unique 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-specific and tissue-specific expression profiles, stability, robust clinical assays for 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 and prognostic tools for GI cancers.

Keywords

Gastrointestinal cancers; Diagnosis; Prognosis; miRNAs

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Introduction

Gastrointestinal cancers refer to malignant conditions of the gastrointestinal tract (GI tract) and digestive tract associated organs including the esophagus, stomach, biliary system, pancreas, small intestine, large intestine, rectum and anus. The GI cancers are collectively the major cause of cancer related morbidity and mortality worldwide [1]. Prognosis of patients with GI cancers remains largely unsatisfactory due to loco-regional recurrence [1]. Despite improvements, the current treatment strategies including surgery, radiotherapy (RT) and/or chemotherapy (CT) have marginally improved the curative expectations and the quality of life of patients; however, the effectiveness of these new tools depends largely on the stage in which tumors can be detected. Therefore, these cancers can be managed by developing biomarkers, which could enhance early detection, improve patient stratification and therapy response prediction resulting in a more favorable disease outcome. Currently, the most important conventional prognostic factors for survival of GI patients are histological tumor grade and tumor stage at the time of diagnosis, including depth of tumor invasion and involvement of regional lymph nodes. In addition to these clinicopathological parameters, biomarkers are being intensively sought and validated for GI cancers. However, low sensitivity and specificity of available biomarkers limit their utility particularly in screening for early stages or in distinguishing aggressive and indolent tumors [2]. Currently, the relative abundance of several proteins is used as an indicator for diagnostic and prognostic potential. However, complexity of protein composition, diversity of posttranslational modifications, low relative abundance of many proteins and difficulties in developing suitable high-affinity detection agents hinder the development of new protein-based biomarkers. This has prompted investigators to identify more specific and sensitive novel markers, which can supplement or complement existing detection methods for better diagnosis and management of lethal GI cancers.

In recent years, miRNAs present in the body fluids such as plasma, serum, urine, saliva, etc. and tissues have been utilized as biomarkers in various diseases including many cancers [3]. These miRNAs are short noncoding RNA molecules of approximately 19–24 nucleotides (nt), involved in post-transcriptional regulation of gene expression. miRNAs bind to the 3' untranslated region of mRNA, which leads to either translational repression, or mRNA degradation initiated by miRNA guided rapid deadenylation [4]. 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 [5–8]. Although hundreds of miRNAs have been shown to be deregulated in cancers, the group of miRNAs actually playing any pathogenic role in cancer has not yet been fully defined [9]. However, recent high throughput screening studies have documented several miRNA expression signatures as promising biomarkers in a wide array of human cancers that are potentially capable of predicting favorable prognosis or exhibit association with tumor development, progression and response to therapy [10–12]. In cancers, approximately half of the deregulated miRNAs have been located near fragile sites, in loci associated with loss of heterozygosity or with DNA amplification, and in common breakpoints [13]. Being physiologically important and deregulated in several malignancies,

miRNAs are considered to carry important information about the pathophysiological status of cancer patients.

While the majority of miRNAs are intracellular, in recent years extracellular circulating miRNAs have been detected in a variety of biological fluids, such as blood, serum and plasma, saliva, urine, breast milk, seminal plasma, tears, amniotic fluid, colostrum, bronchial lavage, cerebrospinal fluid, peritoneal fluid, and pleural fluid [14–17]. Although Lawrie *et al* identified miRNAs from serum of diffused B cell lymphoma patients; it remained unknown whether the miRNAs detected originated from tumor cells or from nonmalignant cell types [18]. The best possible sources of these circulating miRNAs may include not only apoptosis and necrosis of circulating and primary tumor cells, but also immune cells and other blood cells [19]. However, Chen *et al.*, showed different serum miRNA expression profiles among the cancer and the healthy controls suggesting the presence of tumor-specific miRNAs in serum and plasma [20]. It is possible that circulatory miRNAs predominantly originate from apoptotic and necrotic tumor cells and reflect pathophysiology of the underlying disease, thus serving as useful biomarkers to monitor the clinical course of tumors. Initially the stability of miRNAs in body fluids was debatable; however recent studies indicate that circulating miRNAs are present in extracellular vesicles including exosomes, microvesicles and apoptotic bodies, which provide protection from nucleases present abundantly in the body fluids [21, 22]. In addition to vesicle bound miRNAs in body fluids, miRNAs also bind to high density and low density lipoproteins and RNA-binding proteins Ago1 and Ago2.

Overall, tissue specific expression of miRNAs, ease of access in the cell-free body fluids, remarkable stability, sensitive and inexpensive detection supports their potential as disease biomarkers [23–25]. Therefore miRNAs are considered to be attractive candidates as diagnostic, prognostic and predictive biomarkers [26]. Furthermore, a single miRNA can affect several cellular processes, and therefore, successful targeting of miRNAs can potentially provide novel therapeutic avenues to combat malignancies. In this review article, we provide an updated overview of literature and summarize the current knowledge about the diagnostic and prognostic applications of miRNAs in GI cancers.

Esophageal Cancer

Esophageal cancer is the 3rd most common type of cancer among the GI cancers and 6th leading cause of cancer related deaths. In the United States, about 17,990 new cases and 15,210 deaths were estimated in 2013 [27]. The epidemiology of esophageal cancer has changed markedly over the past several decades in the United States. Until the 1970s, squamous cell carcinoma was the most prevalent type of esophageal cancer (90–95%). However due to the lifestyle changes, the incidence of adenocarcinoma has increased intensely in the last two decades [28]. Numerous molecular and histological changes were associated in the multistage conversion of normal squamous epithelium to Barrett's esophagus, low grade and high grade dysplasia and frank adenocarcinoma. Specifically, esophageal adenocarcinoma (EAC) is the most common aggressive tumor that arises from the Barrett's esophagus and Barrett's metaplasia [29]. Hence Barrett's esophagus is the pre-neoplastic condition suitable for identifying and predicting the candidate biomarkers for

early detection and prognostic evaluation. Several studies have highlighted the importance of miRNAs involved during the progression of esophageal cancer [10, 30].

Altered expression of miRNAs during the development of esophageal tumors has been thoroughly investigated during the last decade [30]. Wijnhoven *et al* [31] reported deregulation of 44 miRNAs in the columnar gastric and squamous esophageal epithelium. Furthermore, real time PCR analysis revealed miR-21, miR-143, miR-145, miR-194 and miR-215 to be significantly upregulated in columnar epithelium as compared to normal esophageal squamous tissues. Maru *et al* [32] demonstrated miRNA-196a as a potential marker with dual role in growth acceleration and anti-apoptotic function during the progression from normal esophagus-toBarrett's metaplasia- to dysplasia- to full blown esophageal cancer. Recently, a genome-wide PCR based profiling of 754 miRNAs was evaluated in the sequential stages of esophageal cancer and oncogenic miRNAs such as miR-21, miR-25, and miR-223 and tumor suppressor miRNAs such as miR-205, miR-203, let-7c, miR-133a and miR-133b were found to be aberrantly expressed in Barrett's esophagus and EAC specimens [33]. Tumor suppressor effects of miR-133a have been shown in variety of cancers. Restoration of miR-133a in cancer cells inhibited proliferation, migration, invasion and resulted in G2/S arrest hence validating the tumor suppressive function of miR-133a in cancer cells [33]. Similar tumor suppressor effects have been documented for miR-133b using various cell culture experiments. In silico analysis revealed EGFR and FGFR1 as the predictive targets of 133a and 133b suggesting that these two miRNA's were suggested as potential biomarkers and therapeutic targets for esophageal cancer [33]. Also, a significant down-regulation of miR-375 was exclusively observed in adenocarcinoma tissues indicating this miRNAs as a promising biomarker which can be interpreted with histological grading as well [33]. Other expression profiling studies using next generation sequencing (NGS) in a paired normal vs EAC tissues revealed 26 miRNAs to be frequently deregulated in EAC. Out of 26 deregulated miRNAs, miR-31 and miRNA-31* were extensively identified in the high grade dysplasia and adenocarcinoma specimens [34]. In addition, miR-375 showed remarkable down regulation in EAC [34]. Thus, miRNAs are critical molecular markers that can potentially aid in discriminating the patients who are at high risk of developing adenocarcinoma from the pre-neoplastic lesions of Barrett's esophagus.

Despite aggressive treatment, the clinical outcome remains poor in patients with esophageal cancer. The dismal prognosis of esophageal cancer is mainly attributed to the asymptomatic nature of the disease and late presentation of patients with advanced disease stages i.e mostly detected only after metastasis, making it difficult for surgical removal procedures [35]. Overexpression of miRNA-21 in esophageal tumors was associated with positive lymph nodes and decreased survival of esophageal cancer patients [36]. Significant decrease in cell proliferation and invasion were observed when anti-miR- 21 was overexpressed in human esophageal squamous cell carcinoma cell lines (TE6, TE8, TE10, TE11, TE12, TE14, KYSE30) [36]. In addition, differential expression of miR-375 expression was inversely correlated with pathological stages, metastasis and disease free survival (DFS) of esophageal cancer patients. Importantly, expression of miR-375 was found to regulate genes associated with chemotherapy resistance [37]. Another miRNA miR-143 was found to be

associated with tumor recurrence and invasion in esophageal cancer [38]. Liu *et al* documented the tumor suppressor role of cluster of two miRNAs, miR-143 and miR-145 in esophageal cancer and observed a significant association between risk of esophageal cancer development with silencing of these miRNAs [39]. Several lines of evidence have established that 5q region of the genomic DNA is more frequently lost or deleted in esophageal cancer and human miR-145 and miR-143 were predicted to be associated with 5q33 genomic region [40]. Taken together, miR-145 and miR-143 might be candidate miRNAs for better prognosis of esophageal cancer [39].

Gastric Cancer

Despite three decades of progress in cancer treatment, gastric cancer (GC) remains a significant health problem and second leading cause of death worldwide [27]. Gastric cancer incidence varies dramatically with geographic locations with highest in Eastern Asia (Japan), Eastern Europe, South America and central Asia (Middle East) and lowest in North America [28]. In the USA, GC is the second most common cancer among the GI malignancies with approximately 21,600 new cases and 10,990 deaths estimated to occur in 2013 [27]. The asymptomatic and late onset of gastric cancer practically nullifies medical interventions at the diagnostic stage. However, sequential evolution of histological changes from normal gastric epithelium to gastritis to gastric metaplasia to dysplasia to carcinoma provides a window of opportunity for new detection strategies and newer biomarkers to prevent GC advancement [41]. Therefore, there is a critical need for an early diagnosis based on established markers and alterations in signaling pathways that predispose the gastric epithelial cells for neoplastic transformation.

The involvement of oncogenic and tumor suppressor miRNA in GC is well established [42]. Gastric juice miRNAs are promising biomarkers to detect GC at an earlier stage and several studies have reported the presence of miR-200c, miR-150, miR-342 and miR-378 in the gastric juice as potential candidates for screening gastric cancer patients at earlier stage [43, 44]. Another study using gastric juice from patients with GC, gastric ulcers, gastritis and normal healthy subjects demonstrated significantly lower expression of miR-129-1-3p and miR-129-2-3p in GC patients as comparison with healthy controls [45]. In addition to gastric juice, recently Li *et al.*, reported plasma miRNA-199a-3p as a potential diagnostic marker for early stage GC [46]. A serum based genome wide analysis of patients with GC and healthy controls indicated a unique miRNA signature (miR-1, miR-20a, miR-27a, miR-34 and miR-423-5p) as a novel diagnostic marker for GC [47]. Validation of several miRNAs in a cohort of 60 GC patient and 60 healthy serum samples identified over expression of miR-21 and miR-223 in patients with *Helicobacter pylori* (*H. pylori*) infection as compared with GC patients without *H. pylori* infection [48]. Thus, *H. pylori* infection which is one of the risk factors for GC can also influence the miRNA expression profile in the patient serum. In addition, serum miR-21, miR-146a and miR-148a were also recognized not only as promising biomarkers but are also useful in prediction of lymph node metastasis of GC [49].

In addition to their utility as diagnostic biomarkers, miRNAs can also be used as clinical outcome predictors. Teng *et al* reported the over expression Lin28 as predictive indicator for

locally advanced GC patients undergoing neoadjuvant chemotherapy [50]. In addition, down regulation of miR-206 or overexpression miR-200c are associated with poor prognosis of GC patients [44, 51]. Lower expression of miR-125a-5p is also regarded as independent prognostic indicator for GC [52].

Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the lethal malignancy and the 3rd leading cause of cancer-related deaths worldwide [53, 54]. HCC has extremely poor prognosis with five year survival rate of less than 9% [55]. Therefore, exploration of unexplored molecular mechanisms of HCC will help us to understand its pathogenesis, identify early diagnostic, prognostic markers and therapeutic targets for this lethal cancer.

Unique miRNA expression profiles have been associated with the development, progression and therapy response of hepatocellular carcinoma [56]. Expression profiling of miRNAs in 24 HCC patient tissue samples identified significant upregulation of miR-18, and miR-224 and downregulation of miR-199a*, miR-199a, miR-195, miR-200a and miR-125a compared to their adjacent normal tissues [57]. Several other reports also documented significant down regulation of miR-139, miR-145, miR-214, miR-21, 221, 222, 34a miR-148a, miR-124a-2 and Let-7c and upregulation of miR-9, miR-21, miR-25, miR-137, miR-151, miR-155, miR-182, miR-183, miR-338, miR-219-1, miR-207, miR-185, miR-186 miR-216, miR-221, miR-222, miR-224 and miR-301 [58–60] in HCC tissues and cell lines. A signature of 20 miRNAs was found to differentiate primary HCC from non-metastatic tumors [58]; whereas a set of 40 miRNAs precisely differentiated HCC from cirrhotic tissues. A significant association of miR-222 overexpression with advanced stage and shorter disease-free survival of HCC patients was also observed [61]. Multivariate analysis of upregulated set of miRNAs (miR-155, miR-15a & b, miR-432, miR-486-3p and miR-30b) also showed a significant association with recurrence-free survival in HCC patients [62]. Similarly, expression of miR-15b negatively correlated with risk of HCC recurrence [63]. In yet another study, miR-29c and miR-140-5p were found to be downregulated in HCC patients and associated with poor prognosis [64, 65], suggesting their tumor suppressor role in HCC and indicating their utility as valuable biomarkers for prognosis of HCC. Furthermore, down regulation of miR-140-5p in HCC tissue samples was associated with multiple nodules, differentiation, capsular formation, vein invasion, and shorter overall and disease-free survival of HCC patients [65]. Murakami *et al* reported over expression of miR-18b in HCC patients which associated with shorter relapse-free survival [66]. Furthermore, significant over expression of miR-18b in poorly differentiated HCC was observed [66]. Abnormal expression of miRNAs has also been associated with drug resistance resulting in poor prognosis and reduced patient survival [67, 68]. In addition to their diagnostic and prognostic relevance, expression of miRNAs like miR-26 in HCC patients have been used as an indicator for responsiveness to Interferon (IFN) treatment [56, 59].

Gallbladder Cancer

Gallbladder cancer (GBC) is the 5th most common gastrointestinal cancer [69] with expected 6000 new cases in 2013 as per the estimates of American Cancer Society. Due to

late stage diagnosis, extensive hepatic, lymph-node and vascular metastases, GBC is associated with extremely poor prognosis with overall five-year survival rate of <5% [69, 70]. Chronic inflammation of the gallbladder (Cholecystitis) and gallbladder calcification (Cholelithiasis) are usually considered as conspicuous causative precursors of GBC [71]. Over the years advances have been made to improve the diagnostic and therapeutic modalities (radiation and conventional chemotherapy) to target GBC, yet the prognostic scenario of this lethal malignancy remains as grim as ever [72]. While distant metastases often preclude surgical resection, the extremely high recurrence rates even after complete resection lead to marginal improvement in the overall survival [69]. This necessitates urgent efforts for the identification of reliable diagnostic, prognostic tumor markers and novel cellular targets for curative therapeutic approaches. So far the role of miRNAs in GBC is not very well documented. However, recently a significant overexpression of miR-155 was reported in GBC when compared to normal gall bladder and pancreatobiliary maljunction cases [70]. Up-regulation of miR-155 in GBC cases correlated with poor prognosis of the GBC patients compared to the patients with low miR-155 expression [70]. Further, the study showed the involvement of this aberrantly expressed miRNA in proliferation, aggressive behavior with increased lymph node metastasis and vessel invasion [70]. This study supports the potential of miR-155 as a molecular target in GBC therapy particularly for inhibition of lymph node metastasis and as a prognostic biomarker for GBCs [70]. Moreover, the utility of increased miR-155 expression for distinguishing GBC from benign diseases of gall bladder such as Xanthogranulomatous cholecystitis was also suggested [70]. GBC-specific upregulation with no effect on expression during inflammatory conditions associated with pancreatobiliary maljunction and possibility of its stable detection in serum and/or bile mark it as a prospective novel diagnostic marker for GBC [70]. Molecular studies and clinicopathological analysis of GBCs revealed strong association of aberrantly over-expressed miR-20a with growth, distant metastasis and poor prognosis [73]. It was demonstrated that expression of miR-20a is induced by increased levels of TGF- β 1 in GBC that leads to epithelial to mesenchymal transition (EMT) mediated through inhibition of Smad7 transcription and activation of β -catenin signaling pathway [73]. In addition to the above mentioned miRNAs with oncogenic function, Jin *et al.* have demonstrated that, miR-34a acts as a tumor suppressor in GBC and decreased expression of miR-34a was associated with poor prognosis of GBC patients [74]. Overexpression of miR-34a resulted in inhibition of colony forming ability of CD44⁺CD133⁺ GBC tumor stem like cells *in vitro*. Furthermore, overexpression of miR-34a resulted in reduced xenograft tumors [74].

Various single nucleotide polymorphisms (SNPs) in miRNA genes lead to variations in miRNA expression resulting in diverse functions and, therefore, may represent ideal candidate biomarkers for cancer prognosis. A recent North-India population-based case-control study of GBC explored the function of common single nucleotide polymorphisms (SNPs) in pre-microRNA genes as potential biomarkers for GBC susceptibility [75]. Patients carrying more than two variant alleles due to hsa-miR-146a G>C polymorphism (rs2910164), hsa-mir-196a2 C>T (rs11614913) polymorphism and hsa-mir-499 T>C (rs3746444) polymorphism were associated with increased overall risk of developing GBC with borderline significance. This risk was further accentuated in patients with gallstone disease [75].

Pancreatic Cancer

Pancreatic cancer (PC) is the 4th leading cause of cancer related deaths in the USA with less than 6% five-year survival [27]. Despite extensive efforts in understanding the molecular pathology, PC remains one of the most aggressive forms of cancer due to insufficient knowledge of PC pathogenesis, resistance to conventional therapy, the lack of early diagnostic and predictive markers. The other reason being the late clinical presentation of the disease; approximately 80% of newly diagnosed patients have cancers already metastasized to adjacent organs, for which no curative therapy is currently available. Therefore, better management of PC necessitates the discovery of early diagnostic and predictive markers.

Several studies over the past decade have revealed differential expression of miRNAs in normal pancreas, pre-malignant lesions (PanINs) and pancreatic ductal adenocarcinoma (PDAC) and therefore support diagnostic and prognostic utility of miRNAs [76]. Several miRNA expression profiling studies have revealed PDAC-specific overexpression of miRNAs (miR-376a, miR-301, miR-132 and miR-212) as compared to normal or benign adjacent pancreas [77, 78]. However, Zhang S *et al* demonstrated downregulation of miR-132 in cancer compared to normal and benign tissues [79]. A recent study has reported the downregulation of KRAS targeting miR-96 in PC as compared to normal tissues and demonstrated that the overexpression of miR-96 in PC cells resulted in inhibition of cell proliferation, invasion, induced apoptosis and reduced tumor growth [80]. Ikenaga *et al.* reported overexpression of miR-203 in PC compared to normal pancreas and chronic pancreatitis; and found it to be an independent predictor of poor prognosis in cases with no residual tumors [81]. Expression of miR-216 and 217 and the lack of miR-133a was found to be the only characteristic of the normal pancreatic tissues [82]. Several studies have documented the lower expression of let-7 in PC [83, 84]. A miRNA microarray analysis revealed differential expression of 25 (21 upregulated and 4 downregulated) miRNA distinguishing PDAC from chronic pancreatitis and normal pancreas [82]. Among the 25 upregulated miRNAs, 6 miRNAs namely miR30a-3p, miR-105, miR-127, miR-187, miR-452, and miR-518a-2 were found to be better prognostic indicators in patients with lymph node positive disease. A similar study indicated that a subset of 20 miRNAs could discriminate between PDAC, chronic pancreatitis and normal pancreas [85]. Whereas this study observed overexpression of miR-31, miR-145, and miR-146a in PC cases, another study by Papaconstantinou *et al*, reported downregulation of these miRNAs in PC samples compared to normal pancreas [86]. One possible explanation for this discrepancy might be that the latter study used micro dissected PDAC tissues, whereas the former used the whole PC tissue and most of the time, PC tissues consists 90% of desmoplasia. A similar study by Schultz *et al.*, reported 32 differentially expressed miRNAs between PDAC and chronic pancreatitis, among which miR-148a, miR-196b, miR-196a and miR-205 were already found deregulated in previous other studies [87]. In addition to these studies, over expression of miR-103 and miR-107, and decreased expression of miR-155 were useful discriminators of tumors from the normal pancreas [88]. Munding *et al.*, identified 78 deregulated miRNAs and out of these only miR-135b performed best in discriminating PDAC from Chronic pancreatitis [89]. miR-21 over expression has been shown in 79% of

PDACs and correlated with worse prognosis of the patients [90]. However, no significant correlation of miRNA-21 overexpression was observed with tumor size, differentiation, nodal status or patient T-stage [91]. Another study recently showed upregulation of miR-21, miR-155, miR-210, miR-221 and miR-222 and downregulation of miR-31, miR-122, miR-145 and miR-146a in PCs compared with normal samples of cancer-free individuals [86].

Traditionally, diagnosis of PC is most often done by tissue biopsies and ultrasound guided fine needle aspiration. However, detection of serum and plasma miRNAs can be used as a non-invasive method to determine the progression and extent of metastasis. To this end, Wang *et al* studied the plasma miRNA expression profile of four miRNAs (miR-21, miR-210, miR-155, and miR-196a) in PC patients and observed the differential upregulation of miR-155 and miR-196a, distinguished PC patients from normal healthy controls with a sensitivity of 64% and a specificity of 89%, suggesting that plasma miRNA profiling can be a sensitive and specific blood-based biomarker assay for PC [92]. The expression of miR-18a was also significantly higher in PC samples compared to healthy controls [93]. Similarly, differential upregulation of miR-2 has also been observed in the sera of PDAC patients compared to healthy controls [94]. In addition to plasma/serum, miR-196a and miR-217 are over expressed and detectable in the fine needle aspirates (FNA), and used for diagnosis of PDAC and in discriminating them from chronic pancreatitis [95]. Higher expression of miR-451 and miR-486-5p, and lower expression of let-7c, let-7d, let-7f, and miR-200c in the FNA samples of PC patients was also reported recently [83, 96].

Not only have miRNAs been used as diagnostic markers, they have also been analyzed as prognostic indicators for PC. The first such analysis was done by Bloomston *et al.*, who compared the miRNA expression profile of lymph node positive patients who succumbed to the disease within 2 years and those that survived beyond 2 years. The study reported the association of disease outcome with deregulation of miR-30a-3p, miR-105, miR-127, miR-187, miR-452, and miR-518a-2 [82]. The same study reported the association of poor survival with over expression of 2 miRNAs (miR-196a-2 and miR-219) [median, 14.3 months (95% confidence interval, 12.4–16.2) vs. 26.5 months (95% confidence interval, 23.4–29.6); $P = 0.09$] [82]. In addition, miR-155, miR-203, miR-210, miR-222, miR-203, miR-21 and miR-221 overexpression has also been significantly associated with increased risk of death compared to patients with reduced expression of these miRNAs [81, 96–98]. Using high-throughput Toray's 3D Gene™-miRNA chip, Giovannetti *et al.*, identified miRNA-211 as a single prognostic indicator in resected PDACs [99]. Recently, Schultz *et al.*, observed a significant correlation between overexpression of 2 miRNAs (miR-212 and miR-675) and low expression of miR-148a, miR-187, and let-7g with shorter overall survival (OS) [87, 96, 98]. After calculating prognostic index (PI) for these five miRNAs, the median survival was observed to be 1.09 years [95% Confidence Interval (CI), 0.98–1.43] for $PI > \text{median PI}$ compared to 2.23 years (CI 1.84–4.36) for $PI < \text{median}$. This association was independent of various clinicopathological characteristics of the patient like age, gender, KRAS mutation status, tumor stage, American Society of Anesthesiologists (ASA) score, and differentiation of tumor. Using a cohort of 48 PDAC patient samples, high expression of miR-21 and down regulation of miR-30d was also associated with overall poor

survival [100]. The miR-200 and Let-7 downregulation has also been inversely associated with worsening tumor and patient survival in other studies [83, 83, 96, 96, 98]. From the Let-7 family, in particular a significant downregulation of Let-7b has been observed in human PC cell lines and tissue and associated with PC pathogenesis, apoptosis, and cell growth by targeting several target genes, thereby functioning as a tumor suppressor [83, 98].

Intestinal cancers

A recent study examined the miRNA profile in a cohort of well differentiated neuroendocrine small intestinal tumors, and found that five miRNAs were up-regulated (miR-96, -182, -183, -196a and miRNA-200a), while four were down-regulated (miR-31, -129-5p, -133a, -215) [101]. Since these malignancies are very rare, much needs to be unraveled regarding the precise role of miRNAs in the progression of this cancer.

Despite being the second leading cause of cancer related deaths in the United States [27], most early detection methods for colorectal cancer (CRC) are invasive and/or expensive. The miRNA profile is altered in the tumor tissues, serum as well as in feces of individuals with CRC and several studies have indicated that these deregulated miRNAs could potentially be used both as diagnostic and prognostic indicators of the disease. Several miRNAs including miR-429, miR-17-3p, miR-29a, miR-221, miR-92a and miR-21 have been characterized as possible prognostic markers of CRC [102–106], while miR-150 and miR-126 are being proposed as predictive markers of response to chemotherapy [107]. More specifically, over expression of miR-21 in CRC has been associated with: poor response to adjuvant therapy and more rapid recurrence in stage III patients [108], development of distant metastasis [109], reduced disease-free interval [110], reduced disease free survival in stage II colon cancer patients [111] and worse overall survival [112]. miR-31 was upregulated in CRC and associated with advanced stage and/or worse outcomes [113–115]. In addition, overexpression of miR-141, miR-200c [116] and miR-203 [117, 118] in tumors predicted poor prognosis, either alone or in combination with other miRNAs [119].

Twenty miRNAs were found to be altered in the serum of CRC patients [120]. Based on the miRNA profile prevalent in stage I–II and stage IV patients, it was possible to distinguish CRC patients from a control group [120]. Another study used Real Time PCR to analyze miRNA expression in both CRC tissue and adjacent normal tissue and a panel of colorectal cancer cell lines. It was observed that 13 miRNAs were present at significantly different levels, the most dramatically downregulated were miR-133b and miR-145, while miR-31 (which was also associated with the stage of the cancer), miR-96, miR-135b and miR-183, were significantly up-regulated [121]. Further, higher expression of miR-720 has been reported in CRC tissue compared to normal colon epithelial tissues [121, 122]. Faltejskova *et al.*, independently observed significant downregulation of miR-215, miR-375, miR-378 and miR-422a, and upregulation of miR-135b in CRC tumor tissues compared to adjacent normal tissues [123]. A significant correlation of miR-215 and miR-422a expression with clinical stage was observed [123]. A recent study by Zhang *et al.*, identified 35 differentially expressed miRNAs in stage II colon cancer patients compared to adjacent normal mucosa [124]. Cluster of six miRNAs including miR-21-5p, miR-20a-5p, miR-103a-3p, miR-106b-5p, miR-143-5p, and miR-215 successfully categorized patients into high-risk

and low-risk groups with large differences in 5-year disease-free survival. Yet another study looked at miRNA levels in the fecal samples obtained from both normal volunteers and individuals with colon adenomas and CRC. It was found that miR-21 and miR-106a were elevated in fecal samples from CRC and adenoma patients [125]. It was also demonstrated that certain miRNAs can have prognostic value. For instance, miR-29c expression was found to be decreased in UICC stage II and II patients that have an early relapse in comparison with those exhibiting no early relapse [126]. Further, miR-29c was found to inhibit proliferation and migration as well as cause an accumulation of the G1 and G2 population. Serum miR-29c was observed in these early relapse patients, thus indicating a possible role for this miRNA as a prognostic tool [126]. As in other malignancies, the pattern of expression of miRNAs has been shown to vary during the progression of colon cancer. A panel of miRNAs was shown to contribute to tumor development. The miRNAs miR-10b, miR-210 and miR-708 were found to distinguish primary colon cancer and metastases [127]. The same study identified alterations in a set of interconnected miRNA regulatory networks that governed various cellular processes, including epithelial to mesenchymal transition (EMT). The miRNA miR-10b was found to be linked to patient survival when expressed in metastases [127]. A recent study by Shivapurkar *et al* using serum samples from CRC patients reported significant downregulation of miR-103 and upregulation of miR-596 [128]. Furthermore, the same study reported down-regulated miR-103 in blood samples from CRC patients with disease recurrence compared to recurrence-free patients.

Individual miRNAs have been found to affect disease progression, lending credence to the notion that targeting these miRNAs could be a viable therapeutic strategy in the future. The miRNAs miR-143 and miR-145 were found to act in an opposing fashion, either inhibiting or aiding disease progression [129]. Both miRNAs were found to have a nearly mutually exclusive target gene set. The miR-143 miRNA was found to target DNA methyltransferase 3A in CRC, thereby acting as a tumor suppressor miRNA. Conversely, miR-145 was found to be an oncogenic miRNA, targeting mRNAs involved in cell cycle (*MYC* and *CCND2*) and neuregulin (*MYC* and *ADAM17*) pathways [129]. The miRNA miR-133a was found to be a critical tumor suppressor miRNA, acting in part by regulating levels of the p53 protein [130], by targeting the 3' UTR of the ring finger and FYVE-like domain containing E3 ubiquitin ligase protein (RFFL). This miRNA was found to be down-regulated in primary colon cancer in comparison to surrounding normal tissue.

The *KRAS* and *TP53* mutations are present in a significant proportion of CRC patients (5–20%), and a recent study has indicated that the miRNA let-7a regulates *KRAS* through *TP53* in the *KRAS* mutant HCT116 cells [131]. miRNAs have been found to up-regulate other proteins aiding in CRC progression, such as Fascin1 (*FSCN1*), which is regulated by the miR-451 via the inhibition of AMPK1 and subsequent activation of mTORC1 [132]. Likewise, the important cell cycle regulatory molecule, CDC25A, was found to be regulated by miR-21 [133]. The reduced levels of the miR-224 and the passenger strand of miR-221 were found to reduce the levels of the metastasis suppressor Maspin, thereby promoting the metastasis of CRC [134]. It has also been suggested that the loss of DICER1 function

(involved in miRNA biogenesis) imparts cancer stem cell-like properties to CRC cells, that impacts the biogenesis of several critical miRNAs such as miR-34a and miR-126 [135].

Conclusions and future perspectives

miRNAs play an intrinsic role in the cancer progression, from the initiation of the disease (by regulating levels of critical genes such as *KRAS* and p53) to the advanced metastatic stages of the malignancy. A complex, finely tuned network of miRNAs govern crucial processes such as EMT. The level of miRNAs in feces and serum reflect changes in miRNA expression patterns in the tumor tissues and may be exploited as non-invasive diagnostic and prognostic tools. Given that altering the level of a single miRNA can trigger a cascade of signaling events culminating in a generalized increase/decrease in proliferation, apoptosis, cell growth etc. effective miRNA targeting strategies could yield dramatic results, potentially altering the course of the disease. However, current miRNA targeting technology is hampered by several practical difficulties, primarily, inefficient targeting to the disease-affected organ. Should these significant obstacles be overcome, miRNAs could well be used as prognostic and diagnostic tools as well as viable therapeutic targets. Overall, differentially expressed miRNAs in GI cancers and GI tract associated organs are summarized in Table 1 and Table 2 respectively. These differentially expressed miRNAs are also shown in Figure 1.

Despite significant advances in diagnosis and treatment, morbidity and mortality rates of GI malignancies have remained unchanged over the past decade. The poor diagnostic and prognostic performance of currently used biomarkers mandates identification and evaluation of novel biomarkers with increased sensitivity and specificity. Since their discovery, miRNAs have been assigned important roles and act as master regulators of various physiological processes like metabolism, cell differentiation, stress etc. However, miRNA deregulation contributes to tumorigenesis by augmenting several cellular processes like cell proliferation, migration and invasion. Not only miRNAs have the potential to serve as diagnostic and prognostic markers, they are also being considered as biomarkers for predicting the response to therapy in several malignancies. Their high stability in circulation and formalin fixed tissue samples and relatively inexpensive detection methods suitable for high-throughput analysis makes miRNAs promising candidates for *ex vivo* detection. Due to their tissue and disease stage specific expression, miRNA not only can identify the presence or absence of tumors, they can also determine the primary organ or tissue affected. In addition they can also identify the clinical and pathological stage of the disease. Furthermore, miRNAs can predict risk of progression, relapse, and metastasis, and help to evaluate possible clinical scenarios in relation to the therapy response.

Even though miRNAs have been used as diagnostic and prognostic markers, we still have only a nascent understanding of the role of miRNAs in cancer in general and still face challenges in the research field of tumor-related miRNAs. Use of different methodologies in the miRNA profiling studies have limited the comparability of data and makes it difficult to identify a consistent miRNA signature for diagnosis and prognosis of GI cancers. However, the rapid pace of improvement in miRNA expression profiling technologies and given that new miRNAs continue to be discovered it is likely that future studies will identify useful

expression signatures that may be even more informative for predicting the behavior of GI cancers. Most published studies do not account for the confounding influence of various pathological factors such as hypoxia, infection and cytotoxic treatment [136]. Therefore, to improve the reliability, quality assurance and validity of miRNA signatures, it is recommended to use standardized and more stringent protocols for collecting and analyzing samples. This approach will lead to reduction in inter-study discrepancies and provide highly reproducible results. The clinical validity of these candidate miRNA signatures should be determined using large independent cohorts in multi-centric studies. In combination with the use of more robust platforms, more appropriate bio-computational and statistical analyses should be used to identify candidate miRNA signatures. In addition, use of miRNA signatures along with already established biomarkers for GI cancers may help to better predict the disease outcome on individual patients. Presence of miRNAs in exosomes, microvesicles and/or apoptotic bodies can have different functions during the development of cancer. Much of the current literature describing serum/plasma based miRNA expression profiles does not describe the sub-types of circulating miRNAs, suggesting that future studies concerning circulating miRNAs for diagnostic and prognostic purposes should be focused on the type of circulating miRNAs present in body fluids.

Genetically engineered mouse (GEM) models have been designed to recapitulates histopathological alterations found in human cancers. Integrated human and mouse gene expression analysis of tumor have revealed important similarities [137]. Furthermore, similar miRNA expression profile between mouse p48-Cre/Kras^{G12D} and human PC patients has been reported [138]. Therefore, similar future studies should also be designed using relevant GEM models of other GI cancers to identify pathologically important miRNAs.

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Figure 1.

Important differentially expressed miRNAs in GI malignancies and GI tract-associated organs. These include cancers from esophagus, stomach liver, pancreas, colon, and gall bladder. Upregulated miRNAs are shown in red color whereas the down regulated miRNAs are shown in black color.

Table 1

Differentially expressed miRNAs in gastrointestinal (GI) tract cancers.

miRNAs	Type of Cancer	Status	Identified miRNA Targets	Ref.
miR-205	Esophageal cancer	Up	ZEB1 and ZEB2	[34]
miR-31	Esophageal cancer	Up	KSR2, EMP1 RGS4	[35]
miR-21	Esophageal cancer	Up	cdc25A, PTEN	[33–34, 37]
miR-143, miR-145, miR-133a, miR-133b	Esophageal cancer	Down	FSCN1	[33–34, 40]
miR-375	Esophageal cancer	Down	JAK2 and c-MYC	[34]
miR-143	Esophageal cancer	Down	DNMT3A	[40]
miR-21 and miR-20a	Gastric cancer	Up	PTEN	[36, 40, 46]
miR-129-1-3p, miR-129-2-3p	Gastric cancer	Down	CP110, TOCA1, ABLIM1, SOX2	[46]
miR-199a-3p	Gastric cancer	Up	CD44	[47]
miR-223	Gastric cancer	Up	SH2B3, CDR2, CARM1	[40]
miR-146a	Gastric cancer	Up	SOCS1	[50]
miR-148a	Gastric cancer	Up	DNMT3B	[50]
miR-206	Gastric cancer	Down	BCL2, BDNF	[44–45, 52]
miR-200c	Gastric cancer	Up	TrkB	[44–45, 52]
miR-21	Colon cancer	Up	cdc25A, PTEN, E2F, MYC, NFkB, β -Catenin	[108–112, 125]
miR-596	Colon cancer	Up	LGALS3BP	[128]
miR-31	Colon cancer	Up	KSR2, EMP1 RGS4	[113–115]
miR-200c	Colon cancer	Up	ZEB, CDH1, PLS1, LSR, EPS8L2	[116]
miR-203	Colon cancer	Up	p63, SOCS3, c-jun (AP-1)	[106, 118]
miR-96	Colon cancer	Up	FOXA3a, FOXO1, RAD51, REV1	[121]
miR-720	Colon cancer	Up	TWIST	[121–122]
miR-135b	Colon cancer	Up	LATS2, β -TrCP, NDR2 and LZTS1	[121, 123]
miR-106b-5p	Colon cancer	Up	RBL1 RBL2 and CASP8	[124]
miR-133b	Colon cancer	Down	(MCL-1), BCL2L2, c-Met FSCN1	[121]
miR-143	Colon cancer	Down	DNMT3A	[121]
miR-215	Colon cancer	Down	MDM2, TYMS and SIP1/ZEB2	[123]
miR-378	Colon cancer	Down	IGF1R	[123]
miR-442a	Colon cancer	Down	CYP7A1	[123]
miR-29c	Colon cancer	Down	SIRT1	[126]
miR-103	Colon cancer	Down	DAPK and KLF4	[128]
miR-145	Colon cancer	Down	MYC, CCND2, ADAM17	[125]
miR-375	Colon cancer	Down	p53	[123]

Table 2

Differentially expressed miRNAs in cancers of GI tract associated organs.

miRNAs	Type of Cancer	Status	Identified miRNA Targets	Ref.
miR-155	Gall bladder Cancer	Up	HDAC1, SHIP1	[70]
miR-20a	Gall bladder Cancer	Up	BCL2, PTEN, STAT3, VEGFA, WEE1	[73]
miR-34a	Gall bladder Cancer	Down	NOTCH1, CDC25, CDK6, Bcl2, HDAC1	[74]
miR-18b	Hepatic Cancer	Up	TNRC6B	[67]
miR-23a	Hepatic Cancer	Up	TOP1	[60]
miR-29c	Hepatic Cancer	Down	SIRT1	[65]
miR-140-5p	Hepatic Cancer	Down	TGFBR1 and FGF9	[66]
miR-224	Hepatic Cancer	Down	PPP2R1B	[57,59]
miR-376a miR-301 miR-212 miR-96 miR-21	Pancreatic cancer	Up	CDK2, AGO2 FOXF2, BCC3, PTEN, Col2A1 Rb, PTCH1 FOXA3a, FOXO1, RAD51, REV1 PTEN, P53, NF- κ B, TGFBR2, Tropomyosin 1	[78–79,81–82, 87, 120,100]
miR-105 miR-187	Pancreatic cancer	Up	TLR2, CDK6 DAB2	[83, 88, 97]
miR-148a miR-196b miR-196a miR-205	Pancreatic cancer	Up	DNMT3B, ROCK1 HOXA9/MEIS1, FAS ANXA1 PTEN, PHLPP2	[88]
miR-155 miR-210 miR-221 miR-222	Pancreatic cancer	Up	HDAC1, SHIP1 E2F3, EFNA3, GIT2, MNT, ZNF462, EGF3 E2F, MYC, NFKB, β -Catenin FOS, FOXO3, PTEN, TKB1	[87]
miRNA-211	Pancreatic cancer	Up	CHOP/GADD153	[99]
miR-196a	Pancreatic cancer	Up	HOXA9/MEIS1, FAS	[93]
miR-18a	Pancreatic cancer	Down	KRAS, PIAS3	[94]
Let-7b, let-7c, let-7d and let-7f miR-200c	Pancreatic cancer	Down	KRAS, NRAS ZEB, CDH1, PLS1, LSR, EPS8L2	[84, 97, 100–101]
let-7g	Pancreatic cancer	Down	Collagen type I α 2	[88, 97]
miR-30d	Pancreatic cancer	Down	EZH2, SMAD1, BECN1	[100]
Let-7 miR-155	Pancreatic cancer	Down	RAS, HMG2A, cdc34 APC, FOXO3, MECP2, RUNX2, SMAD1	[84, 85, 89]
miR-122 miR-145 miR-146a	Pancreatic cancer	Down	CUX1, CAT1 SOX9, ADAD17 TRAF6	[87]
miR-200	Pancreatic cancer	Down	FAP	[84, 97]