Circulating MicroRNAs in Colorectal Cancer

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Abstract

Colorectal cancer (CRC) poses a serious threat to the health of global populations. Screening for the early detection of CRC is important to improve patient survival. Circulating microRNAs (miRNAs, miRs), as nucleic acid markers, play a variety of important roles in early screening, diagnosis, TNM stage and prognosis of CRC. In this review, an overview covers recent researches on the roles of circulating miRNAs and their variety potential values correlated with CRC. Studies on the detectable marker values of circulating miRNAs for CRC will help making a global consensus of procedures and standardized protocols to make their clinical transformation more reliable.

Keywords: Colorectal cancer; Biomarkers; Circulating MiRNAs

Introduction

With increasing incidence, colorectal cancer (CRC) remains the second most common cancer in women and the third in men, despite the improved screening techniques, most patients diagnosed CRC are at late stage, CRC is the second leading cause of cancer-related death worldwide [1]. Complete tumor resection by surgery is the main therapeutic modality; it was reported that 5-year survival rates were in range from 93.2% for the earliest stage to 6.6% for advanced disease at diagnosis according to Dukék’s stage. So, good prognosis of CRC is linked to stage at earlier diagnosis. However, due to asymptomatic in early stage of CRC, many cases were detected at late stage. In clinical practice, colonoscopy is used in the diagnosis and treatment of CRC, but has not yet been wide-used as a screening tool because of some limitations, such as the requirements of bowel preparation, cost burden and perforation risk [2]. In addition, fecal occult blood test (FOBT), some convenient circulating blood biomarkers, including the most frequently used marker carcinoembryonic antigen (CEA), carbohydrate antigens, CA125, CA153 and CA199, are lack of sufficient sensitivity or specificity. For example, the specificity of FOBT is about 95%, while the sensitivity of is around 70% to 75% [3], the sensitivity and specificity of CEA is 60% and 34%, respectively [4]. Therefore, new strategies and novel biomarkers with the characters of detection, staging and prediction of outcome are highly desirable and being explored in order to make optimized treatment prescription and to improve prognosis for CRC patients.

A growing number of researches focus on small non-coding RNA molecules, microRNAs (miRNAs, miRs), which play multiple roles in variety of biological processes, including cancer [5-7]. Since 2008, tumor-derived miRNAs were described present stably in circulating blood, as circulating-based markers for cancer detection [8]. Consider the detection of circulating miRNAs have the advantages of simple, inexpensive and noninvasive, combined with the remarkable stability characteristics of mature miRNAs due to the miRNA-Argonaute-protein complex, the discovery of circulating miRNAs as novel biomarkers for cancer intervention has been widely explored [9,10]. Growing evidences have indicated that aberrant expression of circulating miRNAs has an association with cancer including CRC. The diagnostic and prognostic value of circulating miRNAs exhibited in screening and monitoring, predicting recurrence or metastasis including lymph node, vessel, peritoneal invasion or distant metastasis, stratification by TNM stage or even histological differentiation grade, and few are associated with drug resistance, chemoradiosensitivity, tumor size and gender. This review covers recent researches in circulating miRNAs and their variety potential values correlated with CRC (Figure 1).

Experiment Flow

Experiment flow for the identification of CRC-related circulating miRNAs apply step-wise strategy. First experiment samples are selected according to different experimental purpose, microarray-based screening is used for expression profiling. Following is usually the training phase, after an extended scale validation by qRT-PCR, the results obtained from the screening phase are obtained. To the interesting results obtained from the training phase, qRT-PCR is used for further validation, correlation analysis is explored for identifying and verifying target miRNAs relevant to CRC.

Main circulating miRNAs in CRC diagnosis

Early diagnosis and surgical intervention is the most effective treatment and benefit for prognosis improvement of CRC patients. Review of previous studies, we recruited 35 of 46 articles encompassed more than 45 circulating miRNAs, which can be used as biomarker for discriminating CRC patients from control subjects (Table 1). Most miRNAs, including let-7a, let-7g, miR-15b, miR-17, miR-18a, miR-19a, miR-20a, miRNA-21, miR-23a, miR-24, miR-27a-3p, miR-29a, miR-106a, miR-125b, miR-133a, miR-139-3p, miR-141, miR-142-5p, miR-143, miR-152, miR-182, miR-193a-3p, miR-200c, miR-210, miR-221, miR-223, miR-320a, miR-338-5p, miR-372, miR-376c-3p, miR-378, miR-423-5p, miR-431, miR-1229, miR-1246 and miR-1290 were increased in plasma or serum source from CRC, while some miRNAs, including miR-106a, miR-125b, miR-133a, miR-139-3p, miR-141, miR-142-5p, miR-143, miR-152, miR-182, miR-193a-3p, miR-200c, miR-210, miR-221, miR-223, miR-320a, miR-338-5p, miR-372, miR-376c-3p, miR-378, miR-423-5p, miR-431, miR-1229, miR-1246 and miR-1290 were increased in plasma or serum source from CRC, while some miRNAs, including miR-193a-3p, miR-194, miR-601 and miR-760 were decreased in circulating blood of CRC than healthy participants. Interestingly, few miRNAs, such as miR-34a, miR-92, miR-145, miR-150, miR-203, presented contradictory results in different studies.

In a study blood samples of 63 CRC patients and 45 controls were collected, expression of 7 target miRNAs was measured using qRT-PCR, the results showed that miR-34a was significantly reduced in CRC [11].

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![Figure 1: Circulating miRNAs and their variety potential values correlated with CRC.](image)

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Table 1: Potential circulating microRNA biomarkers for CRC.

Meanwhile, the result of a step-wise study yield plasma samples showed miR-34a was significantly up-regulated in CRC [12]. Another miRNA appeared contradictory was miR-92. Based on circulating plasma of mice bearing human colon cancer xenografts, the level of miR-92a was significantly increased [13]. A three-phase (marker discovery; marker selection and validation; large-scale validation) study showed miR-92 is significantly elevated in plasma of patients with CRC [14]. In a study of developing a multi-marker blood based test for diagnosis of CRC, miR-92a was found to be upregulated in pools of plasma samples of CRC patients compared with neoplasm-free controls [15]. Based on 62 paired-plasma samples, using stepwise approach, miR-92a was found over-expressed in CRC and performed best as biomarkers for detecting CRC [16]. Expression of 12 miRNAs in plasma samples was measured using RT-PCR, and the result showed that miR-92a was upregulated in patients with advanced colorectal neoplasia than healthy controls [17]. In a two-phase case-control test designed to identify serum miRNAs as candidate biomarkers for CRC diagnosis, miR-92a was down-regulated in CRC than control samples [18]. The similar contradictory results were also observed in the circulating miRNAs miR-145, miR-150 and miR-203 [19-22].

Values of circulating miRNAs as diagnostic biomarkers

The serum or plasma mRNA (single or panel) to detect CRC yielded an area under the receiver operating characteristic curve (ROC) (AUC) value of 0.67-1.00, with a sensitivity of 47.5% to 100% and a specificity of 41% to 100%, some of which was more sensitive than biomarkers used in clinic, such as serum CEA was reported previously had 60% sensitivity and 34% specificity, the population level use of non-invasive stool DNA tests and the FOBT recommend by guidelines had sensitivities vary from 60% to 95% [23]. CEA, CA125 and CA199 have been reported with the restriction of low specificity and sensitivity, data from a single center study showed that the AUC of serum mRNA for...
Circulating miRNAs in early screening

The above miRNAs in circulating can distinguish between CRC and normal controls. It is widely accepted that CRC mainly develop from precancerous lesions, i.e., polyps and adenomas after many transition stages of malignancy. It has been reported that if CRC patients were diagnosed at an early stage, more benefit would get from curative surgery [26]. As a result, circulating miRNAs aim to screen for the detection of CRC should have an efficiency at delineating the earlier lesions. Combined the above miRNAs, a unique serum or plasma expression profile of miR-15b, miR-17, miR-18a, miR-21, miR-23a-3p, miR-24, miR-142-3p, miR-145, miR-320a, miR-331, miR-376c-3p, miR-423-5p, miR-532, miR-532-3p, miR-652 and miR-1290 could distinguish benign colorectal polyp or adenoma lesions from healthy controls with good accuracy or high AUC values [27-33]. High levels of circulating miR-34a, miR-152 and low miR-150 levels were associated with the distinction of patients with polyps or adenomas from those with CRC (AUC from 0.537 to 0.904) [34]. Distinguish the polyp or adenoma group from the normal group and the benign lesions group is important to improve patient survival and facilitate cancer prevention through the detection and removal of polyps or adenomas.

Circulating miRNAs in TNM stage

Currently, the TNM staging system is remain a useful tool for clinicians, which can be used to estimate tumor burden, guide curative surgical intervention and predict prognosis of patients. Many studies involved circulating miRNAs reported that there was an association between the aberrant expression profiles in circulating blood of CRC and TNM stage. In the previous studies involved in this review, more than 25 circulating miRNAs were reported had an association between clinical TNM stage (including separate analysis of depth of tumor invasion and lymph node status) of CRC and expression levels of circulating miRNAs. These miRNAs contained miR-15b, miR-18a, miR-18b, miR-20, miR-21, miR-23a, miR-29a, miR-29b, miR-92a, miR-96, miR-139-3p, miR-141, miR-152, miR-181b, miR-182, 193a-3p, miR-194, miR-200b, miR-200c, miR-201, miR-338-5p, miR-372, miR-431, miR-601, miR-760 and miR-1290. However, the increasing or decreasing trend closely related with the advancement of cancer TNM I-IV stages existed in circulating miR-21, miR-23a, miR-29a, miR-29b, miR-92a, miR-96, miR-141, miR-182, 193a-3p, miR-194, miR-200b, miR-200c, miR-201, miR-338-5p, miR-372 and miR-1290. The others were associated with either depth of tumor invasion or lymph node status, or between a certain stage and normal.

In terms of the same miRNA, inconsistent results were observed in different studies, such as miR-18a, miR-21 and miR-29a. Expression levels of serum miR-18a and miR-29a were able to distinguish stage III CRC patients from controls in a set of 30 CRC patients and 26 healthy controls [35]. In a study of validation set recruited 224 plasma cases in total from 80 CRC samples and 144 neoplasm-free individuals, higher expression levels of miR-18a, miR-21 and miR-29a were observed in the plasma of CRC samples than in neoplasm-free subjects, but lack of any significant association between the miRNAs and tumor stage [36]. In a study recruited 40 colorectal adenocarcinoma patients and 40 control subjects, expression levels of serum miR-21 were measured using qRT-PCR assay, the results showed high expression level of serum miR-21 was associated with high clinical stages in the patients. Similar results were found in miR-29a in a study recruited 100 sera of patients with CRC and 30 sera of healthy donors [37]. Some miRNAs can distinguish TNM stage in CRC tissue samples, but failed in circulating miRNAs. Take miR-92a as an example, though miR-92a was previously reported to be correlate with TNM stage as to the relationship between the clinicopathologic characteristics and miR-92a expression, based on plasma from 90 patients with CRC, miR-92 levels of individual tumor stage had significant differences when compared to the controls, but it did not vary significantly across the stage in another study [38]. It has been reported that the 5-year survival rate range from 93.2% for stage I to 81.1% for stage IV. Consequently, a reliable treatment plan according to TNM stage may lead to vastly different clinical outcomes [39]. As a more convenient and accurate diagnostic tool, circulating miRNAs have the potential to positively link early diagnosis and treatment of CRC to higher patient survival rates.

Circulating miRNAs related to prognosis

The quality of life is closely related to prognosis of patients with CRC. Besides circulating miRNAs related to TNM staging system, which currently be used as a tool for predicting prognosis and survival. Several circulating miRNAs have been proposed and verified to be used as an important predictive parameter, through which can improve patients’ quality of life by choosing the best combination of treatment modalities, such as surgery, radiation, and/or chemotherapy. Researches about circulating miRNAs independent of TNM stage related to prognosis may focus on predict postoperative survival, early recurrence and metastasis. Kaplan–Meier curve was used for overall survival, relapse free survival and disease free survival analysis according to the levels of circulating miRNAs, such as miR-15b, miR-29b, miR-194, miR-210, miR-221, miR-23a-3p, miR-372, miR-376c-3p and miR-378, aberrant expression led to a worse survival. Based on the difference of preoperative and postoperative expression levels of circulating miRNAs, take miR-24, miR-182, miR-320a and miR-423-5p as examples, circulating miRNAs will be provided with potential prognosis values for CRC progression monitoring after surgery. As important prognostic related factors, metastasis and recurrence are major cause of deaths in patients with CRC, based upon logistic regression analysis, Cox proportional hazards regression analyses and ROC analysis, miR-29a, miR-203, miR-200c, miR-372 and miR-1290 were reported as potential biomarkers. Moreover, the application of pre- and post-operative chemoradiotherapy was also factors influencing the prognosis of patients, circulating miR-20, miR-125b, miR-130, miR-145, miR-216 and miR-372 had been reported to be used as predictive biomarker of the chemoradiotherapy responsiveness.

Discussion

It has been estimated that over 95% CRC patients will benefit from...
radical operation to the cases diagnosed at early stage. Several early
detection procedures by colonoscopy, barium enema, stool- and blood-
based tests have been widely used in clinic, due to the restriction of low
specificity and sensitivity and invasive nature, the minimally invasive
blood based test appears to be more attractive. Circulating miRNAs are
stable owing to protection from endogenous ribonuclease, with the
superiority characteristics of ease to detect, relative noninvasive and
reliable, make these molecules potential to be candidates as biomarkers
to reflect various states in CRC progression [40]. Numerous circulating
miRNAs have been reported and being explored in identifying as
screening, diagnostic and prognostic biomarkers for CRC, but few have
moved into clinical translation and widely available in the clinic, before
entering clinical arena, numerous obstacles need to be overcome [41].

It has been suggested that tumor-derived miRNAs can be present in
circulation based on the variation of circulating miRNAs' expression
level pre- and post-operation, reflect from the expression levels of
circulating miRNAs down-regulated after surgical resection of primary
tumors. However, the expression level of the same miRNA is not always
inconsistent in tissues with that in circulating system. In a study of miR-
141 predicts prognosis as circulating biomarker, plasma level of miR-141
was significantly higher in CRC patients than in controls, but the results
also showed that miR-141 level was not significantly higher in tumor
specimens than in paired adjacent normal controls [42]. It has also been
suggested that miRNAs are secreted from tumor-independent cells,
such as immune and inflammatory cells, which occur coincidentally
at primary lesions, or secreted into body fluids, including blood, urine,
and so on, via be packaged into exosomes [43–46]. To the pathways of
miRNAs into circulating system, several studies demonstrated that
different potential ways, such as direct leakage passive from broken
tumor cell, regulation of chemokines expression and function in tumor
microenvironment, selective activation and secretion, similar to the
release of cytokines and hormones, microvesicle-embedded secretion
[47–50].

Limit the clinical application of circulating miRNAs is also reflected in
the inconsistent results. Tracing it to the cause of these conflicting
conclusions of circulating miRNAs in diagnosis, early screening. TNM
stage and prognosis, may be attributed to the following factors: (1)
genetic variations among different region, ethnic groups and different
environmental and dietary factors, (2) difference of sample collection
procedures or processing conditions, the source choice of plasma
serum or whole blood (3) uniform inclusion and exclusion criteria of
subjects (i.e., early or late stages of cancers) to the same research
purpose (diagnosis or TNM stage), (4) usage of pooled samples, (5)
different internal controls including miR-16, RNU6B or cel-miR-39, and
variety ways of data normalization and analysis in the quantification of
RNA levels with qRT-PCR, (6) different miRNA expression levels between
tissue and plasma, (7) sample size, screening method, and so on [51–56].

Another limitation with the use of circulating miRNAs as biomarkers is
that they are not unique for CRC, but act as broad-spectrum biomarker for
many other cancers or non-oncological diseases, such as lung, gastric,
avian, pancreatic cancers and ulcerative colitis. Therefore, an effective and special diagnostic method should be
considered for the clinical application target CRC.

Conclusion

This review examines issues and advantages of afflicted circulating
miRNAs as biomarkers for CRC. Highlight the clinical significance
need a global consensus of procedures and standardized protocols to
make their clinical transformation more reliable.


