Abstract. microRNAs (miRNAs) are short non-coding RNA sequences that play important roles in the regulation of gene expression. They have significant regulatory functions in basic cellular processes, including differentiation, proliferation and apoptosis. miRNAs are differentially expressed in tumors, compared with normal tissues. Importantly, miRNAs are also stable and abundantly present in body fluids and feces. The high reproducibility, sensitivity and specificity of miRNAs in body fluids and feces enable miRNAs to be used as potential molecular markers for cancer screening. An increasingly large number of research studies have reported the role of miRNAs in this field. In the present review, we focused mainly on the application of detecting miRNAs in stool, sputum, pleural effusion and urine, to detect colon, lung and urological cancers, highlighting the role of miRNAs in early diagnosis and prognosis.

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3. Sputum and pleural effusion miRNA detection in lung cancer
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6. Conclusions and prospects

1. Introduction

Cancer is currently the most lethal human disease. Lung and colorectal cancers are the first and third most common types of cancers and the leading causes of cancer-related mortality worldwide (1). Bladder cancer, a urological cancer, is the second most common malignancy that involves the urinary system, and the clinical outcome is often poor once the tumor becomes invasive (2). Although much progress has been made in the prevention, early diagnosis and treatment of cancer, survival rates are still not optimistic, indicating that a more powerful method to detect cancer in the early stages is needed.

Early detection of cancer has been reported to greatly improve both the survival rate and prognosis, suggesting that the key to oncotherapy may lie in early diagnosis (3-7). Thus, developing a method for the early detection of cancer is both important and necessary. Ideally, an early detection method would have high sensitivity, specificity and repeatability, and would be safe, affordable and acceptable to the patient as well.

Traditional methods, such as colonoscopy (8), bronchoscopy (9) and cystoscopy (10), are used to detect colon cancer, non-small cell lung cancer (NSCLC) and bladder cancer, respectively. These methods have greatly benefited many individuals in the past and they are still used to diagnose cancer. However, their use has been hampered by their invasive nature, the manpower resources they require, their high cost and the discomfort they cause patients (11-13).

Biological screening methods, including the fecal occult blood test (FOBT) for colon cancer; sputum cytology for NSCLC (14); and the bladder tumor antigen (BTA) test, BTA stat test, nuclear matrix protein 22 (NMP22), and urinary cytology for bladder cancer, have also been applied in recent years. However, these methods each have a significant sensitivity (15,16) or specificity (13), but not both.

As previously mentioned, these methods have drawbacks that prevent their wide application. However, miRNA research in recent years has shed new light on early stage cancer detection. miRNAs can function as oncogenes and tumor suppressors (17). Many studies have reported that miRNA levels are altered during cancer (18-20), suggesting that...
miRNA dysregulation may be the perfect tool for the early diagnosis of cancer. miRNAs are short, non-coding RNA sequences of 20-22 nucleotides that are involved in crucial biological processes, such as development, differentiation, apoptosis and proliferation (21-23). Each miRNA has numerous gene targets, and miRNAs mainly function by pairing with the 3'-untranslated regions of target mRNAs (24). Nevertheless, a recent study reported that miR-34a modulates MDM4 expression via a target site in the open reading frame (25). According to existing data, miRNAs regulate at least 30% of protein-coding genes (26) suggesting that miRNAs may control cellular processes in this manner.

This review discusses the possibility of detecting miRNAs in feces, sputum, pleural effusion and urine in order to screen for certain types of cancer, such as colon, lung and bladder cancers, respectively. These three body fluids and stool have been widely used to detect diseases in the clinic for many years, and the results of their biochemical indices have high diagnostic value. These materials have the advantages of reproducibility, abundant content and tissue-specificity.

With the development of genetic sequencing tools, many researchers have realized that traditional clinical detection methods cannot make full use of the genetic value of these materials. Notably, we found that many studies have focused on the potential use of miRNAs in these materials as biomarkers to detect cancer. microRNAs in these materials are useful candidates for cancer detection for the following reason: miRNAs in body fluids and stool are stable under extreme conditions, including a range of temperatures and pH values, after extended storage and after multiple freeze-thaw cycles (27-32), indicating that miRNAs in these materials are stable enough to detect even after the time of collection. However, synthetic miRNAs can be quickly degraded by RNase in the plasma (28). Brase et al (33) hypothesized that miRNAs hide in microvesicles, which protect them against RNase activity, resulting in their stability (Fig. 1). Microvesicles are small particles that are released into the cellular space and blood stream from cell membranes (34,35). Evidence indicates that mRNAs and miRNAs can be transported through microvesicles between cells (36). Furthermore, these encapsulated miRNAs have been found to be involved in the regulation of hematopoiesis and cellular differentiation (37). Microvesicles, also known as exosomes, have been correlated with both cancer stage and miRNA levels in primary cancers when secreted into the extracellular milieu (29,38), suggesting that exosomes can be used to transport genetic information, such as miRNAs, to support tumor growth and progression (39). Additionally, there is another mechanism that can explain miRNA stability. Certain miRNAs have been reported to bind to a specific DNA/RNA-binding protein to avoid degradation (40). miRNAs have been abundantly detected in the stool, sputum, pleural effusion and urine, suggesting that changes in the expression levels of miRNAs can be easily detected. Xie et al (41) showed that miRNAs were more stable than RNA molecules, despite significant miRNA deposition. Evidence has shown that miRNAs can act as oncogenes and tumor suppressors. Therefore, changes in miRNA content may indicate that cancer is present.

In the present study, we summarize the value of miRNAs in three body fluids and stool for the early diagnosis and prognosis of tumors.

2. Fecal miRNA detection in colon cancer screening

Colorectal cancer (CRC) is the third most common cancer worldwide and the leading cause of cancer-related mortality. Approximately 50% of patients will die from the development of distant metastases, and the survival rate over a 5-year period is ~40% after diagnosis and treatment (1). However, early detection of such neoplasms leads to a better prognosis. There are several methods for detecting CRC, but their drawbacks have limited their wide application and dissemination worldwide. Colonoscopy is the gold standard for CRC diagnosis. However, the limitation outlined previously (including the invasiveness of the procedure, the high cost of the equipment and the manpower required), have restricted the wide application of this procedure. Furthermore, clinical guidelines suggest that
miR-144

miR-92a

miR-21

miR-21

miR-135

miR-34b/c

miR-148a

miR-106a

miR-145

miR-143

--- not mentioned in the original study.

miR-92a was detected in the feces of CRC patients, suggesting that it could be a potential diagnostic marker for CRC detection, with a sensitivity of 74% and a specificity of 87% (n=75, P=0.0001) (60). miR-92a and miR-21 were also reported to have these two advantages. miR-92a was found to have a sensitivity of 71.6% and a specificity of 73.3%, whereas miR-21 had a sensitivity of 55.7% and a specificity of 73.3% for CRC (31). Compared with miR-21, miR-92a was able to detect polyps to a greater extent and is likely to be a relevant precancerous polyp marker. The level of miR-92a decreased significantly after the removal of the tumor or advanced adenoma, whereas the level of miR-21 decreased only after the removal of the tumor (31). Link et al (59) reported increased expression of miR-21 and miR-106a in CRC stool samples, compared with normal ones. They used a newly developed DMA (direct microRNA analysis) methodology that easily detected miRNAs in the stool. Kalimutho et al (63) found that promoter methylation of miR-34b/c and miR-148a was detected in the feces of CRC patients, suggesting that miR-34b/c and miR-148a may be involved in colorectal tumorigenesis and metastasis.

3. Sputum and pleural effusion miRNA detection in lung cancer

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. Lung cancer is the leading cause of cancer-
related mortality worldwide (6,64). Therefore, we focused on the application of new miRNA techniques in lung cancer detection. NSCLC can be histologically subdivided into four subtypes: adenocarcinoma, squamous cell carcinoma, large cell carcinoma and 'other' (neuroendocrine cancers, carcinoid tumors) (6). The disease is often diagnosed during the advanced stages and carries a poor prognosis, with a 5-year survival rate of 13% (6,32,65). However, the survival rate of NSCLC increases to 83% when detected during stage I. Many methods are currently used to detect and diagnose lung cancer, including computed tomography, magnetic resonance imaging and bronchoscopy (66). Even though the sensitivities of computed tomography (CT) were reported to be as high as 100% (67-69), the cumulative frequency of subjects with suspicious lesions is high, especially in silicosis patients, in which CT generates a considerable number of false-positive results due to high detection of many non-calcified nodules, which have the potential to be confused with lung cancer (70). These results suggest that although CT is widely used to detect NSCLC, it is plagued by false-positive results at the cost of improved sensitivity (71). Similar to colonoscopy, bronchoscopy is also invasive (12,72). Although sputum cytology is gentle, the low sensitivity limits its wide application. The levels of bronchial epithelial cells, which are detected by sputum cytology, are very low in the sputum (14).

Aside from traditional methods, many studies have reported the use of biological methods, such as molecular genetics, to screen for lung cancer that may meet the standards for an ideal diagnostic method. It has been reported that tissue-based biomarkers can distinguish the tumors which originate in the lung from metastases that originated in other sites in the body by detecting significant biomarkers, such as tumor-suppressor genes, regions of chromosomal amplification, differential miRNA expression and variable miRNA expression (73-78). However, this method is limited by the accessibility of the specimens and the stability of the assessment offered. Blood-based biomarkers are another biomarker method that can be used to detect lung cancer. Indeed, blood is an ideal material due to the abundance of cancer-specific biomarkers, such as DNA methylation (79), gene expression (80), blood-miRNA (81), CTC (82) and cell-free DNA. Unfortunately, there are still some drawbacks preventing blood-based biomarkers from being successful clinical biomarkers of cancer, such as low sensitivity, scarce quantities of any given marker, the complex nature of the blood matrix and lack of reproducibility (73). One study attempted to detect specific DNA in the sputum to screen for lung cancer but did not detect any differences in either the free DNA or cellular DNA concentrations in the sputum of lung cancer patients compared with that of healthy controls (83), indicating that DNA in the sputum may not be an effective biomarker for lung cancer.

Many miRNAs have been proven to be abnormally expressed in cancer tissue (57). For this reason, miRNAs are potentially a useful tool for diagnosing and screening human malignancies, including lung cancer (78). Sputum in particular has been considered to be a potential surrogate material for the non-invasive diagnosis of lung cancer. Taken together, these results indicate that miRNAs in the sputum may be used to screen for lung cancer. miRNAs in the sputum can be detected using real-time RT-PCR with TaqMan miRNA assays (Applied Biosystems) (6,32). Sputum miRNAs are very stable (6,32), similar to the stool miRNAs mentioned above. The combination of sputum miRNAs have shown promising results (Table II). miR-21 has been found to be overexpressed in many types of cancer and this finding has been demonstrated in many studies (84). In two recent studies, miR-21

<table>
<thead>
<tr>
<th>Materials</th>
<th>miRNA</th>
<th>Refs.</th>
<th>Dysregulation</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
<th>Samples</th>
<th>Normalization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>miR-21</td>
<td>(31)</td>
<td>Upregulated</td>
<td>100</td>
<td>69.7</td>
<td>40</td>
<td>RNU6B</td>
</tr>
<tr>
<td></td>
<td>miR-155</td>
<td>(31)</td>
<td>Upregulated</td>
<td>--</td>
<td>--</td>
<td>40</td>
<td>RNU6B</td>
</tr>
<tr>
<td></td>
<td>miR-486</td>
<td>(6)</td>
<td>Downregulated</td>
<td>79.4</td>
<td>66.9</td>
<td>72</td>
<td>RNU6B</td>
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<tr>
<td></td>
<td>miR-126</td>
<td>(6)</td>
<td>Downregulated</td>
<td>73.8</td>
<td>67.2</td>
<td>72</td>
<td>RNU6B</td>
</tr>
<tr>
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<td>59.5</td>
<td>72</td>
<td>RNU6B</td>
</tr>
<tr>
<td></td>
<td>miR-21</td>
<td>(6)</td>
<td>Upregulated</td>
<td>79.2</td>
<td>72.6</td>
<td>72</td>
<td>RNU6B</td>
</tr>
<tr>
<td></td>
<td>miR-182</td>
<td>(6)</td>
<td>Upregulated</td>
<td>79.5</td>
<td>64.3</td>
<td>72</td>
<td>RNU6B</td>
</tr>
<tr>
<td></td>
<td>miR-200b</td>
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<td>Upregulated</td>
<td>78.5</td>
<td>62.9</td>
<td>72</td>
<td>RNU6B</td>
</tr>
<tr>
<td></td>
<td>miR-375</td>
<td>(6)</td>
<td>Upregulated</td>
<td>80.6</td>
<td>63.9</td>
<td>72</td>
<td>RNU6B</td>
</tr>
<tr>
<td>Malignant effusion</td>
<td>miR-93</td>
<td>(75)</td>
<td>Downregulated</td>
<td>--</td>
<td>--</td>
<td>184</td>
<td>ath-miR159a</td>
</tr>
<tr>
<td></td>
<td>miR-100</td>
<td>(75)</td>
<td>Upregulated</td>
<td>--</td>
<td>--</td>
<td>184</td>
<td>ath-miR159a</td>
</tr>
<tr>
<td></td>
<td>miR-134</td>
<td>(75)</td>
<td>Downregulated</td>
<td>--</td>
<td>--</td>
<td>184</td>
<td>ath-miR159a</td>
</tr>
<tr>
<td></td>
<td>miR-151</td>
<td>(75)</td>
<td>Downregulated</td>
<td>--</td>
<td>--</td>
<td>184</td>
<td>ath-miR159a</td>
</tr>
<tr>
<td></td>
<td>miR-345</td>
<td>(75)</td>
<td>Downregulated</td>
<td>--</td>
<td>--</td>
<td>184</td>
<td>ath-miR159a</td>
</tr>
<tr>
<td></td>
<td>miR-24</td>
<td>(40)</td>
<td>Upregulated</td>
<td>80.5</td>
<td>53.6</td>
<td>40</td>
<td>ath-miR156a</td>
</tr>
<tr>
<td></td>
<td>miR-30d</td>
<td>(40)</td>
<td>Upregulated</td>
<td>67.1</td>
<td>71.4</td>
<td>40</td>
<td>ath-miR156a</td>
</tr>
<tr>
<td></td>
<td>miR-26a</td>
<td>(40)</td>
<td>Upregulated</td>
<td>--</td>
<td>--</td>
<td>9</td>
<td>ath-miR156a</td>
</tr>
</tbody>
</table>

‘--‘ not mentioned in the original study.
was reported to have a sensitivity of 72.6% and a specificity of 79.5% (32) and a specificity of 69.66% (95% CI, 0.46-0.86) and specificity of 100.00% (95% CI, 0.77-1.00) (32). Therefore, examination of miR-21 expression had higher sensitivity than that of sputum cytology (47.82% (95% CI, 0.27-0.69) sensitivity and 100.00% (95% CI, 0.77-1.00) specificity (32)) for the diagnosis and early detection of lung cancer in patients. In addition, it has been reported that increased miR-21 expression is not significantly associated with length of smoking exposure in both cancer patients and controls, suggesting that dysregulation of miR-21 in lung cancer might not be caused by tobacco smoking-related damage (32). Even though overexpression of miR-155 may not distinguish lung cancer patients from controls (32), it is correlated with shortened survival of patients after resection (78). In other words, elevated miR-155 may indicate poor prognosis in lung cancer. Yu et al (6) reported that detection of a combination of different miRNAs (miR-486, miR-21, miR-200b and miR-375) may be a better predictor, with a sensitivity and specificity of 80.6 and 91.7%, when compared with that of a single miRNA (as shown in Table II). Xing et al (65) also showed that detection of a combination of miRNAs (miR-205, miR-210 and miR-708) greatly improve sensitivity and specificity. These two studies indicate that the future detection of miRNAs may involve the detection of a combination rather than a single miRNA. In addition, Yu et al (6) found that miRNA markers had higher diagnostic efficiency for adenocarcinomas than for squamous cell carcinomas of the lung.

Pleural effusion is tightly correlated with NSCLC. Approximately 15% of cancer patients are diagnosed with malignant pleural effusions (MPEs) during early diagnosis (85). MPEs are an important route of proliferation of tumor cells and are a frequent cause of morbidity in NSCLC in lung cancer (85). MPEs are very crucial for the treatment of NSCLC. Not all patients benefit from chemotherapy, particularly those with short overall survival times (86). There are many methods that can be used to detect MPEs, including cytology, needle biopsy and medical thoracoscopy. Cytology is the standard diagnostic method for malignant effusions. Malignant cells are used as a diagnostic sign, but the quantity of malignant cells may be rather low, limiting the rate of positives (~50-70%), even with repeated testing (87). Although needle biopsy and medical thoracoscopy can improve the sensitivity of diagnosis, their invasiveness and high cost limit their wide use (88). Many studies have reported using biomarkers to detect pleural effusion, such as marker proteins (89), DNA methylation status (90) and cell-free mRNA levels (91), but these methods are limited by their diagnostic accuracy. Research personnel have noticed the close relationship between miRNAs and cancer, thus, they attempted to find evidence that could demonstrate that pleural effusion miRNAs are novel biomarkers for lung cancer diagnosis and early detection (92). However, to the best of our knowledge, few studies have been conducted in this new field of interest. Xie et al (41) demonstrated that the levels of miR-30d, miR-24, miR-26a are higher in malignant effusions compared with normal effusions. miR-152 was first found to be a potential diagnostic biomarker for drug sensitivity since the amounts of miR-152 in tumor cells that were resistant to docetaxel were lower than those of chemosensitive tumor cells (41). Wang et al (86) reported five miRNA expression signatures (high expression levels of miR-100 and low expression levels of miR-134, miR-345, miR-151 and miR-93) that were an independent prognostic marker of poor survival. This was the first report of miRNA expression signatures in MPEs that predicted NSCLC patient prognosis. It seems that using miRNAs as a biomarker to screen for MPE is a promising strategy, yet the mechanism remains unknown. Thus, further study is warranted. In conclusion, detection of miRNAs in the sputum and pleural effusion is a promising method that may be used to prevent lung cancer, both by early detection and accurate prognosis.

4. Urine miRNA detection in urological cancer

Bladder cancer is the second most common malignancy of the urinary system. These tumors are often invasive at the time of diagnosis (2). Urothelial carcinoma (UC) is among the five most common malignancies worldwide, and it is also the second leading cause of mortality in patients with genitourinary tract malignancies (93). UCs are the most common histological type of bladder cancer. Ninety-five percent of primary urothelial cell cancers arise from the bladder.

There are several clinical methods that are used to detect bladder cancer. Cystoscopy is currently the standard diagnostic tool, but it is difficult for cystoscopy to detect flat lesions or carcinoma in situ. In addition, the invasive nature and high expense of the procedure restrict it from being widely used (94,95). Urinary cytology may be a useful method for the detection of bladder cancer, due to the non-invasive nature and high specificity of the procedure (90-95%); however, it has a rather low sensitivity (30-40%) (96). Therefore, many alternative methods have been presented to diagnose bladder cancer, such as the BTA test, BTA stat test and NMP22 (97-99). These methods have a higher sensitivity (50-70%) than cystoscopy, but the increased sensitivity comes at the cost of specificity (60-80%) (95). Even though many achievements have been made in prevention and treatment in recent years, the rates of morbidity and mortality remain high (100). A new biomarker for bladder cancer detection is urgently needed.

As previously discussed, miRNAs are aberrantly expressed or mutated in many types of cancers, suggesting that detection of aberrant miRNA in the urine may be a useful method for bladder cancer screening. In addition, miRNAs have been reported to be stable in the urine and also show high specificity and sensitivity (95,101-103). These characteristics indicate that urinary miRNAs are a potential biomarker for bladder cancer and UC screening. It has been reported that patients with bladder cancer have lower expression of miR-200 family members (miR-200a, miR-200b and miR-200c), miR-192 and miR-155 in the urinary sediment, and higher expression of miR-155 in the urinary supernatant (101). It was also shown that the levels of these miRNAs were altered after surgery. The postsurgical levels of miR-200a, miR-200b, miR-200c, miR-141, miR-429, miR-205, miR-192 and miR-146a increased significantly, whereas the level of miR-155 remained similar (101). Taken together, these results suggest that bladder cancer is the direct cause of depressed urinary miRNA levels, but the mechanism of this suppression is unknown. This study also revealed reverse correlations between the expression of miR-200 family members and EMT markers (ZEB1, vimentin, TGF-β1 and
miRNA Refs. Dysregulation Specificity (%) Sensitivity (%) Samples Normalization

miR-143 (93) Downregulated -- -- 37 miR-16
miR-222 (93) Upregulated -- -- 37 miR-16
miR-452 (93) Upregulated -- -- 37 miR-16
miR-96 (85) Upregulated 89.2 71 149 RNAU6B
miR-183 (85) Upregulated 77.3 74 149 RNAU6B
miR-200a-b-c (91) Downregulated (urinary sediment) 52.6 (mir-200a) 100 (mir-200a) 75 β-glucuronidase and RNU48
miR-192 (91) Downregulated (urinary sediment) -- -- 75 β-glucuronidase and RNU48
miR-192 (91) Downregulated (urinary supernatant) -- -- 75 β-glucuronidase and RNU48
miR-155 (91) Downregulated (urinary sediment) -- -- 75 β-glucuronidase and RNU48
miR-155 (91) Upregulated (urinary sediment) -- -- 75 β-glucuronidase and RNU48

'--' not mentioned in the original study.

5. Common methods used in miRNA diagnosis

The potential of miRNAs in four materials (stool, sputum, pleural effusion and urine) to serve as biomarkers for cancer screening was discussed above. miRNA diagnostic methods are varied (110). In this section of the study, we will focus on the steps that need to be taken to obtain miRNAs and the methods that are used to detect them.

To obtain miRNA profiles, the following steps need to be taken: sample collection, miRNA extraction, miRNA detection, data processing and statistical analysis. Each step is important to the final result. Sample collection is particularly important as it determines the reliability of the results. In this step, researchers should consider many factors, such as age, ethnic group, gender and prior treatments (111). Concerning miRNA extraction, miRNAs can be isolated from samples using three pre-methods: miRNeasy, TRIzol and mirVANA (112). Even though all three methods are suitable for profiling miRNAs from total RNA, researchers still need to be prudent in choosing a method, since small differences exist among the methods and may be a source of bias. miRNA detection is based on the expression levels of miRNAs that have been demonstrated to play a role in disease (111). Researchers should choose the appropriate technology to detect miRNAs using the various available methods. In addition to the detection itself, the stability and reproducibility of the method should also be taken into consideration, to reduce the deviation (113). Data processing mainly refers to the pre-processing of miRNAs for detection and normalization (111), which is necessary to minimize systematic experimental or technical variations. Statistical analysis is the last step and mainly focuses on comparing the differences between groups and indicating the characteristics of the miRNAs in the urine are summarized in Table III.

RhoA (101). Downregulation of miR-200 family members facilitates EMT of the transitional epithelium and promotes cancer progression (104). This may explain the mechanism, but further study is required. In other studies, miR-452 and miR-222 were reported to play an oncogenic role, while miR-143 was able to function as a tumor suppressor (103,105-107). The present study also revealed that miR-452 may contribute to tumorigenesis and aid in bladder cancer diagnostics, whereas miR-143 and miR-222 may be related to tumor progression and may be used for clinical outcome assessment (103,107). In addition, expression levels of of miR-222 and miR-452 were inversely correlated with ERBB4 expression, while ERBB4 was localized to several cellular counterparts, including the membrane (108), cytoplasm (108) and nucleus (109). miR-222 was correlated with ERBB3 protein expression (103), which is also related to tumor stage, grade, size, growth pattern, recurrence, disease-specificity and overall survival (103). Although the study did not reveal the translocation mechanisms of ERBB3 and ERBB4 in bladder cancer progression, it did reveal the clinical relevance of subcellular protein localization (103), providing new insight into the relationship between miRNA, protein and bladder cancer.

In UC, miR-96 and miR-183 levels in patient urine samples were found to be significantly higher than those of the control group, with 71.0% sensitivity and 89.2% specificity, and 74.0% sensitivity and 77.3% specificity, respectively. However, more false-positive cases were found in miR-183 detection compared with miR-96 detection, suggesting that miR-183 may be useful as a staging marker but not as a diagnostic marker. miR-183 is upregulated and functions in UTI as well as UC, and miR-96, which has a high sensitivity and specificity, seems to be a tumor biomarker that can be used to distinguish UC patients from non-UC patients (95). The present study also showed that 9 genes involved in activating apoptosis were commonly downregulated in both miR-96 and miR-183 transfectants (95). The
probability that the differences are clinically relevant, using the Student's t-test as many studies have reported (113,114).

Although miRNA detection methods vary, the vast majority of them rely on Watson-Crick base-pairing between complementary chains of nucleotides and hybridization between a strand of nucleic acid and its target miRNA (111). We summarized the advantages, disadvantages and improvements of 6 of the most widely used methods: Northern blot analysis, bioluminescence, RT-PCR, fluorescence correlation spectroscopy, in situ hybridization and microarray (115-132) (Table IV).

### 6. Conclusions and prospects

In the present review, we discussed the possibility of screening miRNAs in the stool, sputum, pleural effusion and urine to distinguish colon cancer, NSCLC and bladder cancer. Other body fluids contain miRNAs as well, such as amniotic fluid, cerebrospinal fluid, colostrum, peritoneal fluid, plasma, saliva, seminal fluid and tears (133). Few studies have reported the relationship between cancer and miRNAs in amniotic fluid, cerebrospinal fluid, colostrum, saliva, peritoneal fluid, seminal fluid and tears. Although many studies have reported the close relationship between plasma miRNAs and cancer, one plasma miRNA has been shown to be altered in multiple types of cancers [e.g. changes in miR-21 in the plasma can potentially indicate colorectal cancer (134) and gastric cancer (135)], which makes the diagnostic value lower than the four materials we listed previously. We did not put much emphasis on the blood biomarker limitations we discussed (low sensitivity, scarce quantity, complex nature and lack of reproducibility). In addition to miRNAs in the blood, a recent study also reported that miR-421 in gastric fluid could be used as a biomarker to screen for gastric cancer, with a sensitivity and specificity that were equal to 71.4 and 71.7%, respectively (136). This study also demonstrated that miRNAs in gastric fluid had superior purity to miRNAs in the plasma. However, it is not easy to obtain gastric fluid in clinical practice and more research on miRNAs in gastric fluid is still needed. Thus, we did not focus on the application of miRNA detection in gastric fluid.

Biomarkers are important for the early detection and prevention of malignancies as they are altered before histological and morphological changes occur. The ideal biomarker must be non-invasive, inexpensive, specific and sensitive to the disease state and a reliable early indication of disease before clinical symptoms appear (133). Even though many biomarkers (most of them are protein) have been used to screen for cancer, they do not function as expected. Improving the diagnostic specificity and sensitivity of proteins is expensive, time-consuming and difficult (133). Meanwhile, miRNA detection is much easier due to PCR or other DNA amplification methods, which can compensate for the low content limits, indicating that miRNAs may be promising biomarkers for screening cancer.

miRNAs are excellent biomarker. The specificity and sensitivity of miRNAs for screening cancer are higher than other biomarkers, particularly the combination of specific miRNAs, as we have previously discussed (Tables I-III).

### Table IV. Features of the common methods used in miRNA diagnosis.

<table>
<thead>
<tr>
<th>Method</th>
<th>Refs.</th>
<th>Advantage</th>
<th>Limitation</th>
<th>Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern blot analysis</td>
<td>(105-108)</td>
<td>Gold standard for miRNA expression profiling</td>
<td>Poor sensitivity</td>
<td>The use of locked nucleic acid (LNA)-modified oligonucleotide probes (107)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High specificity</td>
<td>Time-consuming</td>
<td></td>
</tr>
<tr>
<td>Bioluminescence</td>
<td>(109)</td>
<td>Rapid and high-sensitivity</td>
<td>Complex steps</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Suitable for application in clinical diagnosis</td>
<td></td>
<td></td>
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<tr>
<td>RT-PCR</td>
<td>(110,111)</td>
<td>High sensitivity and accuracy</td>
<td>Expensive</td>
<td>Use of LNA-modified primers (112)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Easy to operate</td>
<td>Low throughput</td>
<td>Use of quantitative stem-loop RT-PCR for the detection of mature miRNAs (110)</td>
</tr>
<tr>
<td>Fluorescence correlation</td>
<td>(113)</td>
<td>High sensitivity</td>
<td>Special equipment is needed</td>
<td>Use of a dual probe labeling system (113)</td>
</tr>
<tr>
<td>spectroscopy</td>
<td></td>
<td>Low detectable concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In situ hybridization</td>
<td>(114-116)</td>
<td>Specific to the type of cell</td>
<td>Low quantification power</td>
<td>LNA miRNA oligo probe (116)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Semiquantitative analysis</td>
<td>Low throughput</td>
<td>Use of RNA molecules act as a primer (117)</td>
</tr>
<tr>
<td>Microarray</td>
<td>(118-120)</td>
<td>High throughput</td>
<td>Lack of quantitative data</td>
<td>Probe design</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Widely used</td>
<td>Expensive equipment</td>
<td>Sample labeling</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Immobilization chemistry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Microarray chip signal-detection method (121,122)</td>
</tr>
</tbody>
</table>
Detection of miRNAs is also an inexpensive and rapid method that costs ~$10 (US) and takes ~3.5 h to obtain the results (95), indicating its possible use to detect tumor biomarkers. In addition, it is a non-invasive method for screening cancer and the materials are easy to obtain.

However, even with all of these advantages, more effort is required to clarify the usefulness of miRNAs. Recent research has focused on the phenomenon of aberrantly expressed miRNAs in related body fluids and feces. The mechanisms of action remain unknown, suggesting that the mechanisms require future investigation. In addition, it is not easy to identify miRNA target genes. A single miRNA may regulate the transcription of more than one mRNA, and one specific mRNA may be regulated by several miRNAs (103,137), making it difficult to determine a particular miRNA. In many studies, RNU6B was reported to be an endogenous control that could be used to normalize the expression of miRNAs in tissue specimens, but to the best of our knowledge, it may not be the ideal endogenous control for miRNAs due to its rapid degradation in samples (such as stool) (138) and uncertain changes in the content (95). Therefore, it is important to find a stable powerful endogenous control. Although several studies have proposed solutions (59,95), further examination is required.

In conclusion, miRNA detection is a promising method for cancer screening. Many opportunities and challenges lie ahead. We believe that miRNA-based detection will be used for cancer screening in the near future.

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References

MiRNA expression in human cancer: a systematic review.


