MINI-REVIEW

Circulating miRNAs: Promising Biomarkers of Human Cancer

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Abstract

Background: With the development of technologies to look at the expression levels of hundreds of miRNAs at a time and the clear role of miRNAs in cancers, groups began looking at miRNAs profiles of different cancers, especially the circulating miRNAs. We intended to make sure whether circulating miRNAs could be a promising biomarker of human cancers. Method: We comprehensively searched the Cochrane Library, Medline and EMbase from 1966 to Nov 2009 for the following terms: ("miRNA" or "microRNA") and ("tumor" or "carcinoma") and ("plasma*" or "serum" or "circulating"). Detailed information was extracted from studies that met the inclusion criteria: blood-based miRNAs in human cancers and studies published in the English literature. Results: The current review show that different researches use different measurement methods which might impact the results; Cancers treatment might have an affect on circulating miRNAs; some miRNAs are multi-faceted RNA; small sample size might produce selection bias. Furthermore, because of the lack of randomized controlled trials and the heterogeneous nature of the available data, no attempt was made to perform quantitativemeta-analyses. Conclusions: In this review, based on those researches, circulating miRNAs are promising and difficulties for their future application for diagnosing human cancers.

Keywords: Human cancers - miRNAs - biomarkers

Asian Pacific J Cancer Prev, **12**, 1117-1125

Introduction

Cancer is the third leading cause of mortality after cardiovascular and infectious diseases in the world. Although we have made great advances in the understanding of cancer biology and pathogenesis as well as in the development of new targeted therapies, the progress in developing improved early diagnosis and screening tests has been inadequate. As a result, most cancers are diagnosed in advanced stages, which lead to poor outcomes. Now, intense research is focused on seeking specific molecular changes that are able to identify patients with early cancer or precursor lesions. Biological samples such as blood, serum, stool, pancreatic juice or urine, as well as both DNA and RNA, have been analyzed for tumor-specific changes. However, due to the simplicity of getting a blood sample, easily testable biomarkers found in blood serum would be especially useful. Furthermore, the unique patterns of disordered miRNA expression in each type of cancer, their stability in serum (Chen et al., 2008; Mitchell et al., 2009) and their role as biomarkers of disease risk due to inherited polymorphisms suggest that miRNAs may potentially serve as novel molecular biomarkers for clinical cancer diagnosis.

MiRNAs are non-coding, single-stranded RNAs of approximately 22 nucleotides and constituted a novel class of gene regulators that are found in both plants and animals (Bartel 2004). Bioinformatics approaches for identifying miRNAs rely on evolutionarily conserved sequences(Bentwich 2004). It has been reported that miRNAs are critical in development of organisms (Ambros, 2003; Chen et al., 2004),differentially expressed in tissues (Xu et al., 2003), involved in viral infection processes (Pfeffer et al., 2004), and associated with oncogenesis (Calin et al., 2002; Calin et al., 2004). In the past few years, several reports related important aspects of miRNAs in tumor cell lines and tissues. Here, we focused on the potential use of miRNAs as diagnostic biomarkers of human cancer in serum-based screening.

Search Strategy and Selection Criteria

1966 to Nov 2009 for the following terms: ("miRNA" or "microRNA") and ("tumor" or "carcinoma") and ("plasma*" or "serum" or "circulating"). Detailed information was extracted from studies that met the inclusion criteria: blood-based miRNAs in human cancers and studies published in the English literature.

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Circulating miRNAs in Human Cancers

It is reported that miRNAs could be an ideal class of blood-based biomarkers for cancer detection because: (i) miRNA expression is frequently dysregulated in cancer (Calin and Croce, 2006; Esquela-Kerscher et al., 2006), (ii) expression patterns of miRNAs in human cancer appear to be tissue-specific (Lu et al., 2005) and (iii) miRNAs have unusually high stability in formalin-fixed tissues (Li et al., 2007; Xi et al., 2007). This third point led us to speculate that miRNAs may have exceptional stability in plasma and be promising biomarkers for diagnosing human cancers.

Leukemia

MiRNAs play a very important role in normal hematopoiesis because they regulate hematopoietic differentiation in almost every stage, while their aberrant expression has been associated with many diseases, including hematological malignancies (Vasilatou et al., 2009). Increasing evidence has shown that miRNAs could function as either tumor suppressors or oncogenes in cancers such as leukemia, while other miRNAs might be beneficial for diagnosis and prognosis, predicted to be newly developed biomarkers.

Acute lymphoblastic leukemia: a malignant disorder of lymphoid progenitor cells, affects both children and adults, with peak prevalence between the ages of 2 and 5 years. The precise pathogenetic events leading to development of acute lymphoblastic leukaemia are unknown (Pui et al., 2008). Therefore, it is hard to diagnose leukaemia in the early stage. Recently, it has been reported that miRNAs are circulating in serum (Chim et al., 2008, Gilad et al., 2008) and tumor-derived miRNAs such as miR-155, miR-21, miR-15b, miR-16 and miR-24 are detected in the plasmas and serums of tumor patients (Lawrie et al.,2008; Mitchell et al., 2008). These might be a new class of effective biomarkers, and we expect that the miRNAs abundance profile in plasma might reflect physiological and/or pathological conditions.

There is only one article reporting circulating miRNAs in acute lymphoblastic leukaemia. In 2009, Tanaka et al (2009) performed miRNA microaray to obtain insight into miRNA deregulation in the plasma of a leukemia patient. They have revealed that miR-638 is stably present in human plasmas, and miR-92a dramatically decreased in the plasmas of acute leukemia patients. Especially, the ratio of miR-92a/miR-638 in plasma was very useful for distinguishing leukemia patients from healthy body. Furthermore, the author supposed that the ratio of miR-92a/miR-638 in plasma has strong potential for clinical application as a novel biomarker for detection of leukemia.

Chronic lymphocytic leukemia: This accounts for around 30% of leukaemia cases in the United States of America, with the highest incidence in the elderly population (Chiorazzi et al., 2005). The age-adjusted incidence rate of chronic lymphocytic leukaemia is 4.0 per 100,000 men and women per year. Clinically, Chronic lymphocytic leukemia is a heterogeneous disease. Consistent with clinical heterogeneity, a number of molecular prognostic factors have been defined for B cell chronic lymphocytic leukemia, most notably mutation status of the IGHV locus, ZAP-70 expression and recurrent cytogenetic lesions (Chiorazzi et al., 2005). More recently, miRNAs have also been proposed to have prognostic and pathogenic significance.

Thirteen miRNAs associated with prognostic factors were firstly revealed in chronic lymphocytic leukemia (Calin et al., 2005). Among them, miR-15a and miR-16-1 have been clearly demonstrated to function as tumor suppressors. MiR-15 and miR-16 are located on chromosome 13q14, a region deleted in more than half of B cell chronic lymphocytic leukemia patients, and were found either absent or down-regulated in the majority (~68%) of chronic lymphocytic leukemia patients (Calin et al., 2002). Reduced expression of miR-29b and miR-181b are also associated with poor prognosis in chronic lymphocytic leukemia. Pekarsky et al. (Pekarsky et al., 2008) determined that TCL1 expression is regulated by miR-29b and miR-181b. TCL1 is an oncogene and high levels of TCL1 are associated with high levels of ZAP-70 and unmutated IgVH in chronic lymphocytic leukaemia (Herling et al., 2006, Yan et al., 2006). Thus, miR-29b and miR-181b function as tumor suppressors by targeting the oncogene TCL1 and are used as diagnostic and prognostic markers in chronic lymphocytic leukemia treatment. However, few clinical trials refer to circulating miRNAs in chronic lymphocytic leukemia.

Acute myeloid leukaemia: few studies have correlated miRNA expression changes with prognosis in acute myeloid leukaemia. Garzon et al (2008) first reported that in a relatively older AML cohort of patients (median age 59) with intermediate- and poor-risk cytogenetics, patients with high expression of miR-199a and miR-191 had a significant shorter overall survival and EFS. These results were validated in a second cohort of 60 acute myeloid leukaemia patients with similar characteristics using a different technology; qRT-PCR. These two miRNAs (miR-191 and miR-199a) predicted outcome independent from other variables, including age and cytogenetics. Marcucci et al (2008) more recently showed an miRNA signature that correlated with EFS in young acute myeloid leukaemia patients with high-risk molecular features. This subgroup of AML represents more or less a third of young acute myeloid leukaemia patients. The prognostic signature included miR-181a and miR-181b which were inversely correlated with risk of event and miR-124, miR-128-1, miR-194, miR-219-5p, miR-220a, miR-320 which were positively associated with the risk of event. This prognostic signature was independent from other factors. Garzon et al (2008) studied intermediate- and poor-risk cytogenetic groups, whereas Marcucci et al (2008) studied samples of leukemia cells from adults under the age of 60 years) focused on high-risk acute myeloid leukaemia. Till now, no article could give us the information on circulating miRNAs in acute myeloid leukaemia.

Hodgkin lymphoma: There are a few studies referring to miRNAs alterations in Hodgkin Lymphoma. Kluiver et al. (2005) analyzed Hodgkin lymphoma cell lines and

tissue samples and detected high levels of BIC and mir-155 in Hodgkin lymphoma. Thereafter, the first description of circulating miRNAs that had potential as non-invasive diagnostic markers in Hodgkin lymphoma for was made in 2008 when Lawrie et al investigated tumor-associated miR-155, miR-210 and miR-21 in serum of diffuse large B-cell lymphoma (Hodgkin lymphoma) patients and healthy controls (Lawrie et al., 2008). They showed that circulating miRNAs were clearly detectable in serum samples and that higher levels of specific miRNAs were associated with diagnosis and prognostic outcome in Hodgkin lymphoma patients.

MiRNAs are highly significant not only physiological processes but also in pathological processes and tumorigenesis. To date, miRNAs expression profiles in many types of cancers have been identified and miRNAs expression signatures associated with types and cytogenetics in leukemia have also been reported. However, further investigation is needed regarding how the disregulated miRNAs participate in leukemogenesis and how the aberrantly expressed miRNAs are regulated. Although the pathogenesis in leukemia is still unclear, additional studies on the roles of miRNAs will provide new insights concerning the complicated gene regulated network and shed light on novel strategies for the diagnosis of leukemia.

Lung cancer

With more than 215,000 new cancer cases and more than 160,000 cancer deaths estimated in 2008(Jemal et al., 2008), lung carcinoma continues to be the leading cause of cancer mortality in the United States. Despite potentially curative surgery, about 40% of patients will relapse within 5 years (Hoffman et al., 2000). Cancers, including lung cancer and colorectal cancer, are often diagnosed at a late stage with concomitant poor prognosis (Duffy, 2001, Thomas and Sweep, 2001, Duffy, 2007, Roulston, 1990). Although tumor markers greatly improve diagnosis, the invasive, unpleasant, and inconvenient nature of current diagnostic procedures limits their application (Duffy, 2007, Roulston, 1990). Hence, there is a great need for identification of novel non-invasive biomarkers for early tumor detection.

A large number of researches have found that significant differences were presented between the exosomal miRNA levels for the lung adenocarcinoma group and the control group(Yanaihara et al., 2006, Lebanony et al., 2009, Raponi et al., 2009). In 2006, Yanaihara et al (Yanaihara et al., 2006) found that miRNA expression profiles might be diagnostic and prognostic markers of lung cancer. The authors focused on 12 specific miRNAs study and had found elevated in lung cancer, including miR-21 and miR-155. High expression of miR-155 has correlated with significantly shorter survival, and low expression of let-7a-2 has conferred poor prognosis in resected lung adenocarcinoma. Intriguingly, in 4 lung adenocarcinoma cases in which paired tumor and plasma samples were examined, there was a close correlation between circulating miRNAs of tumor-derived exosomes and tumor miRNAs, confirming that miRNA expression in peripheral blood could be a surrogate of miRNA expression in the tumor biopsy. Thereafter, several reports show that exosomes could be an important resource of cell-free miRNA in serum or plasma(Rabinowits et al., 2009, Rosell et al., 2009). In 2009, Rabinowits et al(Rabinowits et al., 2009) reported that it was different in total exosome and miRNA levels between lung cancer patients and controls, and the similarity between the circulating exosomal miRNA and the tumor-derived miRNA patterns. Therefore, the author suggested that circulating exosomal miRNA might be useful as a screening test for lung adenocarcinoma. Furthermore, Rosell et al(Rosell et al., 2009) suggested that the expression of specific circulating miRNAs is a good surrogate of tumor miRNA expression initiates a new paradigm that will be useful not only for early diagnosis but also for prognostic and therapeutic decisions.

Ovarian cancer

In 2008, it was expected that 20,180 women will be diagnosed with ovarian cancer and 15,310 will succumb to the disease (Jema et al., 2008). Ovarian cancer is a devastating illness in which only 20% of patients are diagnosed with stage I disease (Cannistra, 2004). The poor prognosis associated with ovarian cancer is multi-factorial; a lack of minimally invasive, early detection tests, subtle symptom development and tumor chemo-resistance. Even with the advent of chemo-resistance assays it is still difficult to predict drug resistance and only 10–15% of patients will remain in prolonged remission after initial cytotoxic therapy. While annual pelvic examination is widely practiced, it lacks the sensitivity to be used a screening strategy for ovarian cancer (Myers et al., 2006). Women at high risk for ovarian cancer may typically undergo screening with trans-vaginal ultrasound and serum CA-125. CA-125, however, remains a poor marker for early stage disease with a documented sensitivity of 40% (Jacobs and Menon,2005, Jacobs et al., 1993). In recent years, molecular biomarkers have been increasingly investigated for cancer diagnosis and prognosis. The emergence of molecular diagnostic techniques brings new tools to individualized cancer patient care (Bast and Hortobagyi, 2004). One major category of molecular cancer markers that holds promise is based on gene expression studies.

Multiple recent profiling studies also indicate that miRNA expression is significantly changed in ovarian cancer (Yang et al., 2008, Iorio et al., 2007, Yang et al., 2008, Dahiya et al., 2008, Nam et al., 2008). For example, Nam et al reported that miR-200 was upregulated in ovarian cancer and higher expression of miR-200 was associated with poor prognosis (Nam et al., 2008). Yang et al(Yang et al., 2008) thought that let-7i might be used as a therapeutic target to modulate platinum-based chemotherapy and as a biomarker to predict chemotherapy response and survival in patients with ovarian cancer. While, miR-9 and miR-223 can be of potential importance as biomarkers in recurrent ovarian cancer(Laios et al., 2008). Recently, it has been demonstrated that the miRNA signature of circulating tumor exosomes of ovarian cancer patients demonstrates high correlation with miRNA expression of the primary tumor (Taylor et al., 2008). Thereafter, Resnick et al(Resnick et al., 2009) used 28

serums of epithelial ovarian cancer patients to determine the utility of serum miRNAs as biomarkers for diagnosis. MiRNAs-21, 92, 93, 126 and 29a were significantly over-expressed in the serum from cancer patients compared to controls. MiRNAs-155, 127 and 99b were significantly under-expressed. So, the author suggested that the serum of individuals diagnosed with ovarian cancer is feasible(Lodes et al., 2009).

Breast cancer

Breast carcinoma, which is the second most prevalent cancer in women, is diagnosed in >200,000 woman in the USA every year. Early detection is a major factor contributing to the 2.3% annual decline in breast cancer death rates over the past 10 years (Weir et al., 2003). Nonetheless 40,480 women in the USA were projected to die from breast cancer in 2008 (Society, 2008), in part because currently available breast cancer screening tools such as mammography and breast examination miss 10-40% of early breast cancers and are least effective in detecting cancer in young women, whose tumors are often more aggressive. An invasive needle or surgical biopsy must be performed when an area of suspicion is identified in order to confirm, by cytologic or histologic evaluation, the presence of malignancy, even though 66–85% of abnormalities are benign (Fahy et al., 2001).

To date, only two markers have been established so far in the routine assessment of breast cancer: ER (for predicting response to endocrine therapies) and HER2 (for predicting response to Trastuzumab) (Thompson et al., 2008). Although these markers are currently available, ER and HER2 assessment is far from perfect (Piccart-Gebhart et al., 2005). A number of circulating tumour markers (e.g., carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3)) are used clinically in the management of breast cancer, but the sensitivity of these markers is low, so that they are not useful as screening tools (Harris et al., 2007) though they have long been in clinical use as prognostic markers and to monitor for disease progression or recurrence.

Alterations in miRNA expression have been associated with tumor suppression or tumorigenesis, metastasis and poor prognosis in human breast cancer. Numerous studies suggest that miRNA expression signatures in tumor tissues may become useful biomarkers in diagnosis and prognosis of breast cancer(Iorio et al., 2008, Lowery et al., 2008, Shen et al., 2008). Hoffman et al(Hoffman et al., 2009) confirmed that miR-196a-2 might have a potentially oncogenic role in breast tumorigenesis, and the functional genetic variant in its mature region could serve as a novel biomarker for breast cancer susceptibility. Sempere et al(Sempere et al., 2007) found that miR-145 has been considered to have potential clinical applications as a novel biomarker for breast cancer diagnosis. Yang et al(Yan et al., 2008) suggested than miR-21 may serve as a molecular prognostic marker for breast cancer and disease progression. Until 2009, Zhu et al(Zhu et al., 2009) measured miRNA in the serum of samples with and without breast cancer. They found: 1) miRNA species can be detected in archived serum; 2) miR-155 may be differentially expressed in the serum of women with hormone sensitive compared to women with hormone insensitive breast cancer. Therefore, the author suggested screening serum for miRNAs that predict the presence of breast cancer is feasible, and may be useful for breast cancer detection. However, the study had been limited by small numbers. This concept needs extensive investigation to validate the theory.

Colorectal cancer

Colorectal cancer is the third commonest malignant neoplasm worldwide and the fourth leading cause of cancer-related deaths worldwide (Shike et al., 1990). It is a significant health problem in the UK, accounting for 22.9 deaths per 100,000 in men and 14.2 deaths per 100,000 in women in 2005(Aslam et al., 2009). The overall 5-year survival rates for colonic and rectal cancers diagnosed from 1999 to 2004 were 49.6% for men and 50.8% for women, and the estimated 10-year survival in 2008, calculated using a hybrid approach, for patients diagnosed with disease in 2000-2001, was 45 percent (Rachet et al., 2008). So, there is a need to improve early detection screening methods for colorectal cancer. If detected early, colorectal cancer is highly curable. Colonoscopies are a reliable and accurate screening tool but their high cost and invasiveness lead to reduced screening rates. On the other hand, faecal occult blood tests (FOBTs) are less invasive, less expensive, have even been shown to reduce deaths caused by colorectal cancer(Hewitson et al., 2008); but the limited sensitivity and specificity of FOBT make it a less than ideal screening method for detecting colorectal cancer. Many researchers are trying to identify non-invasive screening methodologies to improve early detection of colorectal cancer to reduce the health burden of this disease. Blood-based miRNAs offer a prospect of developing a simple blood test which could be used for detection of tumors and differentiation of adenomas from colorectal cancers in patients referred to surgical outpatient clinics.

More than 100 circulating miRNAs can be identified in the blood of healthy individuals (Mitchell et al., 2008) and this profile differs significantly from that of patients with colorectal cancer who have several tumor-specific miRNAs. Chen and colleagues (Chen et al., 2008) demonstrated 69 miRNAs in the serum of patients with colorectal cancer that were not present in the serum of healthy controls. Moreover, they identified a unique expression profile of 14 serum miRNAs for colorectal cancers that were not present in another cancer group (lung cancer). Recently, using quantitative real-time polymerase chain reaction (RT-PCR), Ng and colleagues examined the expression levels of miRNAs in plasma from a series of colorectal cancer patients and controls (Ng et al., 2009). In a training population of 25 colorectal cancer patients and 20 healthy controls, the expression levels miR-17-3p and miR-92 were found to be elevated in the plasma of colorectal cancer patients. They then analysed a validation cohort of 90 colorectal cancer patients and 50 healthy controls and found that the expression of miR-92 in plasma could distinguish colorectal cancer patients from healthy control patients with 70% specificity and 89% sensitivity. MiRNAs contribute to carcinogenesis and may provide

new therapeutic strategies for cancer, but it is too early to know if expression of levels of circulating miRNAs will be of sufficient diagnostic value for their use as a screening method for colorectal cancer(Schetter and Harris, 2009). Until now, this is the first clinical trial that has demonstrated some potential for miRNA expression in plasma to be used as a diagnostic biomarker for colorectal cancer, but many issues will have to be addressed before these findings can be translated into a clinically useful, noninvasive screening strategy for colorectal cancer. These results will have to be replicated in multi-centre, large sample randomized controlled trials. If these results are valid, prospective studies must evaluate the performance of this test to determine a realistic performance expectation for this test.

Esophageal cancer

Esophageal adenocarcinoma has the fastest increasing incidence of any solid tumor in the United States (Pohl et al., 2005) and the 5-year survival rate for this disease is dismal, ranging from 14-22%(Gamliel et al., 2005). While the incidence of esophageal squamous cell carcinoma has been declining or has remained constant in the United States, that of esophageal adenocarcinoma has increased >300% in the past 30 years and continues to rise (Chang et al., 2004). Dysplasia alone as a marker of malignant progression is mired by pathologists' differential interpretation of degrees of dysplasia(Reid et al, 1994). As only a subset of dysplastic lesions will progress to cancer, the challenge lies in sub-stratifying biopsies based on the probability of malignancy development. If molecular markers of premalignancy can be identified on small pieces of esophageal tissue obtained endoscopically, this may lead to improved early detection and provide objective criteria for selection of patients who may benefit from aggressive surgical treatment. Recently, miRNA expression profiles unique to esophageal cancer histologic types, Barrett's status, and survival have been uncovered (Feber et al., 2008, Guo et al., 2008), albeit limited samples sizes.

miRNA expression profiles in esophageal adenocarcinoma were first reported by Feber et al. (2008). MiRNA expression of 35 frezen specimens (10 adenocarcinomas, 10 squamous cell carcinomas, 9 normal epithelium, 5 Barret's esophagus and 1 high grade dysplasia) was analyzed using Ambion bioarrays containing 328 human miRNA probes. They found that mir-203 and mir-205 was expressed 2-10 fold lower in squamous cell carcinomas and adenocarcinomas compared with normal epithelium. Mir-21 expression was 3-5 folds higher in both tumors versus normal epithelium. In 2009, Mathe found miR-21, miR-223, miR-192, and miR-194 expression was elevated, whereas miR-203 expression was reduced in cancerous compared with noncancerous tissue in adenocarcinoma patients (Mathe et al., 2009). So, the author thought miRNA expression profiles distinguish esophageal tumor histologies and discriminate normal tissue from tumor MiRNA expression may prove useful for identifying Barret's esophagus patients at high risk for progression to adenocarcinomas. However, few clinical trials has focused on circulating miRNA in Esophageal cancer. Further investigation is required to determine if a circulating miRNA biomarker can detect esophageal cancer.

Liver cancer

Liver cancer is common globally, with dismal outcomes and an increasing incidence in the United States (Parkin et al., 2005). To date, surgery remains the most effective treatment with curative potential. However, only about 10 to 20% of patients with hepatocellular carcinoma are currently eligible for surgical intervention (Ji et al., 2009). Therefore, it's dramatically needed to find a sensitive biomarker to detect liver cancer at the early stage.

Liver cancer development is thought to develop in a multi-step process requiring the accumulation of several structural and genomic alterations and affecting many different pathways (Thorgeirsson et al., 2002; Roessler et al., 2007). It has been suggested that many of the miRNA changes that occur during hepatocarcinogenesis do so early, so that many changes that predispose to liver cancer have already taken place in liver cirrhosis and other premalignant lesions (Jiang et al., 2008). This was confirmed by Wang et al. (2009). They found specific circulating miRNAs could be detected significantly earlier after liver injury. In 2006, Kutay et al (Kutay et al., 2006) firstly reported that downregulation of miR-122 could be a potential biomarker for liver cancer in rodent and human hepatocellular carcinomas. Thereafter, Lodes et al (Lodes et al., 2009) studied the evaluation of miRNA expression patterns in human serum for five types of human cancer, prostate, colon, ovarian, breast and lung, using a pan-human miRNA, high density microarray. They show that sufficient miRNAs are present in one milliliter of serum to detect miRNA expression patterns, without the need for amplification techniques. In addition, the author suggested that it was able to use these expression patterns to correctly discriminate between normal and cancer patient samples. Furthermore, few article reported circulating miRNAs in liver cancer detection.

Renal cancer

During the past 2 decades, the incidence of the cancers of the kidney and renal pelvis, the vast majority of which are renal cell carcinomas (RCCs) has increased by approximately 2% per year. 5-year survival ranging of late stage RCC is limited from 5% to 10% for lack of efficacy in chemotherapy and radiation therapy (Bukowski, 1997). The clear cell histology type, renal clear cell carcinoma (RCCC), is approximately 80-85% of metastatic RCC and the most frequent subtype of RCC. Histopathology of these tumors has been correlated with distinctively different genetic changes, indicating that unrelated molecular mechanisms underlie the development of each type of tumor (Kovacs, 1993).

Altered levels of miRNAs have been reported in a variety of human cancers, including RCCC(Murakami et al., 2006). Gottardo et al(Gottardo et al., 2007) researched 20 kidney carcinomas specimens by ologonucleotide microchips and found a set of 4 human miRNAs (miR-28, miR-185, miR-27, and let-7f-2) in 245 miRNAs were

found significantly upregulated in renal cell carcinoma compared to normal kidney. While, two years later, Huang et al(Huang et al., 2009) reported that 2 (let-7g and miR-21) upregulated miRNAs of 81 miRNAs were identified valid expression in RCCC samples. Recently, Yi et al(Yi et al., 2009) believed 38 miRNAs exhibited higher expression in the renal cell carcinoma samples than that in the normal control. Circulating miRNAs have also been shown to be predictive of malignancy and survival in renal cell carcinoma patients (Feng et al., 2008). However, further long-term researches are required to investigate the relationship between miRNAs and renal carcinoma as well as their expression in plasma.

Other cancers

Furthermore, in patients with squamous cell carcinoma of the tongue, plasma levels of miR-184 were significantly higher than those in healthy individuals, and miR-184 levels were significantly reduced after surgical removal of the primary tumors (Wong et al., 2008). Moreover, miR-21, known to be over expressed in gliobalstoma tumors (Skog et al., 2008), was elevated in serum microvesicles from glioblastoma patients. There is no related clinical trial in melanoma, pancreatic cancer,

Can Circulating miRNAs really predict human cancers?

Different measurement methods could impact the results

An example of published data from two different miRNA expression profiling techniques that do not show strong agreement is illustrated in the following comparison. Schetter et al (Schetter et al., 2008) labeled colon cancer tissue miRNAs by reverse transcriptase extension with a labeled primer and hybridized the target to a microarray, while Monzo et al (Monzo et al., 2008) determined colon cancer tissue miRNA expression levels by TaqMan RT-PCR. When the 26 upregulated miRNAs from the Schetter study are compared to those from the Monzo study, only 14 miRNAs are in agreement (54%). In a similar comparison of up-regulated prostate cancer tissue miRNAs from studies by Tong et al (TaqMan data (Tong et al., 2009)), Porkka et al (array data (Porkka et al., 2007)), and Ambs et al (array and TaqMan data (Ambs et al., 2008)), very little overlap of miRNA expression data can be seen: little or no overlap in data between Tong et al and Porkka et al, and only 1 of 33 and 1 of 34 miRNAs were in agreement for Tong et al compared to Ambs et al, and for Porkka et al compared to Ambs et al, respectively. Of the 15 unregulated serum miRNAs, Lodes et al(Lodes et al., 2009) report for prostate cancer, 4 are in agreement with data from Ambs et al, and 2 are in agreement with data from Porkka et al. therefore, systematic measurement techniques should be building for the future clinical research.

Cancers treatment might have an affect on circulating miRNAs

We do not yet know the affects of gender, age and cancer treatment on miRNA levels in serum. Radiation and chemotherapies that result in remission of cancer should also result in a change in the serum miRNA profiles. Wong, et al. (Wong et al., 2008) have shown that plasma levels of miR-184 were elevated in patients with squamous cell carcinoma of the tongue, and that plasma miRNA levels were reduced after surgical removal of the tumor. This would indicate that cancer treatment does have an affect on the levels of cancer-specific miRNAs in circulation. The samples used in those studies were from cancer patients that were treated by chemotherapy in most cases. Because we do not have detailed information on the results of treatment on cancer progression or remission we cannot include this variable in our analysis. Additional studies with well-documented patient samples will be needed to address this question.

Some miRNAs are multi-faceted RNA

More than 1000 miRNAs are expressed in human cells, some tissue or cell type specific, others considered as house-keeping molecules. Functions and direct mRNA targets for some miRNAs have been relatively well studied over the last years. Every miRNAs potentially regulates the expression of numerous protein-coding genes (tens to hundreds), but it has become increasingly clear that not all miRNAs are equally important; diverse high-throughput screenings of various systems have identified a limited number of key functional miRNAs over and over again. Particular miRNAs emerge as principal regulators that control major cell functions in various physiological and pathophysiological settings. MiR-21 has been identified as the best hit in a number of medium-scale and high-scale profiling experiments designed for the detection of miRNAs dysregulated in cancer (Krichevsky and Gabriely, 2009). In a large-scale profiling of miRNA expression in 540 human samples derived from 363 specimens representing six types of solid tumours and 177 respective normal control tissues (Volinia et al., 2006), miR-21 was the only miRNA up-regulated in all types of the analysed tumours, including the breast, colon, lung, pancreas, prostate, and stomach. Additional studies demonstrated elevated miR-21 expression in hepatocellular carcinomas, gastric cancer, ovarian cancer, cervical carcinoma, multiple head and neck cancer cell lines, papillary thyroid carcinoma and some other solid tumours. More recent studies indicate that miR-21 is also up-regulated in leukaemic cancers.

Small sample size might produce selection bias

Although these results demonstrate the possible application of miRNA-associated SNPs in cancer diagnosis, one must be prudent in interpreting the data on account of the small sample size. Therefore, multi-center, large, independent, well-characterised, family and population-based case–control and additional validation studies are warranted.

Others

Even though diverse studies have reported the stability of miRNAs in serum even under severe denaturing/degrading conditions, several topics still need to be refined further. First, we are missing large studies reporting miRNA levels in plasma and serum from hundreds of normal individuals of both genders and various ages, so

we do not know if specific miRNA levels are the same in young women and old men, for example. Second, some studies have demonstrated that the endogenous controls used at present, such as RNU6B, in miRNA expression studies are degraded in serum; therefore, to obtain accurate and reproducible results, we need new, more robust standardization methods. Finally, we need to gain a better understanding of the mechanisms by which miRNAs are released in plasma/serum. That is, does this occur by cellular destruction or does an active secretory mechanism really exist? Certainly, the future will bring more details, which will allow us to solve the puzzle of miRNA detection in various types of body fluids; and this will bring a new era to the field of diagnostic markers for human cancers.

5. Limitations

Because of the lack of randomized controlled trials and the heterogeneous nature of the available data, no attempt was made to perform quantitativemeta-analyses. In the absence of standard criteria for the quality assessment of laboratorybased, observational studies on miRNA and heterogeneity of outcome measures included in this narrative review, no quality assessment of included studies was carried out. Therefore our review may have some bias.

6. Conclusion

Therefore, based on those researches, circulating mi RNAs are promising and difficulties for their future application for diagnosing human cancers. Still, large, independent, well-characterized, family and population-based case–control and additional validation studies are warranted to determining whether circulating miRNAs could serve as biomarkers of human cancers.

Acknowledgements

The authors declare no competing financial interests. Jingqiu Cheng, Yanrong Lu, Ya Dai have made substantial contributions to conception and design; Jinjing Wang, Dongliang Li, Changguo Wang, Fang Xue, Bo Qin acquisition of data, or analysis and interpretation of data; Yuchuan Huang and Jie Zhang have been involved in drafting the manuscript or revising it critically for important intellectual content; Yanrong Lu and Ya Dai have given final approval of the version to be published. All authors read and approved the final manuscript.

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