Full Length Research Paper

Circulating micro ribonucleic acids (miRNAs): promising biomarkers for human lung and liver cancers

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Micro ribonucleic acids (miRNA) is released and circulated in the blood of cancer patients. Changes in the levels of circulating nucleic acids have been associated with tumor burden and malignant progression. In the past decade, lots of information on the potential use of circulating nucleic acids for cancer screening, prognosis and monitoring of the efficacy of anticancer therapies has emerged. We intended to make sure whether circulating miRNAs could be a promising biomarker of human cancers. We comprehensively searched the Cochrane Library, Medline and EMbase from 1966 to July 2011, using 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 lung or liver cancers and studies published in the English literature. The current review showed that different researches used different measurement methods which might impact the results; Cancers treatment might have an effect 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 quantitative meta-analyses. In this review, based on those researches, circulating miRNAs are promising and difficulties for their future application for diagnosing human lung or liver cancers.

Key words: MiRNAs, lung cancer, liver cancer, biomarker.

INTRODUCTION

Micro ribonucleic acids (miRNAs), the 17- to 25-nucleotide-long non-coding RNAs, regulate expression of approximately 30% of the protein-coding genes at the post-transcriptional level and have emerged as critical components of the complex functional pathway networks controlling important cellular processes, such as proliferation, development, differentiation, stress response' and apoptosis. Abnormal expression levels of miRNAs, regulating critical cancer associated pathways have been implicated to play important roles in the oncogenic processes, functioning both as oncogenes and as tumour suppressor genes.

With the continuous improvement of biologic techniques, microRNAs can be efficiently isolated and evaluated from tumor tissue samples even after formalin fixation and paraffin embedding (Yan et al., 2008), indicating that they are remarkably stable. In 2008, two research groups (Mitchell et al., 2008; Chen et al., 2008) first reported that serum and plasma contain a large amount of stable microRNAs, and that the expression of these microRNAs showed great promise as novel noninvasive fingerprints for the early diagnosis of various cancers and other diseases. Both research teams have clearly demonstrated that, serum microRNAs are

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Abbreviations: RT-PCR, real-time polymerase chain reaction; **miRNAs,** micro ribonucleic acids; **NSCLC,** non-small-cell lung carcinoma.

resistant to RNase digestion and other harsh conditions such as boiling, extremes of pH, extended storage, and freeze-thaw cycles. Mitchell et al. (2008) demonstrated that serum miR-141 can distinguish patients with prostate cancer from healthy controls. Employing Solexa and quantitative real-time polymerase chain reaction (RT-PCR), Chen et al. (2008) identified specific expression patterns of serum microRNAs for lung cancer, colorectal cancer, and diabetes. Mitchell et al. (2008) demonstrated that microRNA levels in plasma and serum were strongly correlated, indicating that both will be suitable for investigations of microRNAs as blood-based biomarkers.

Search strategy and selection criteria

We comprehensively searched the Cochrane Library, Medline and EMbase from 1966 to Jul 2011 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 lung or liver cancers and studies published in the English literature.

Circulating micro ribonucleic acids (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 was frequently dysregulated in cancer (Esquela-Kerscher and Slack, 2006; Calin and Croce, 2006), (ii) expression patterns of miRNAs in human cancer appeared to be tissue-specific (Lu et al., 2005), and (iii) miRNAs had unusually high stability in formalin-fixed tissues (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.

Circulating micro ribonucleic acids (miRNAs) in 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 would relapse within 5 years (Hoffman et al., 2000). Cancers, including lung and colorectal cancer, are often diagnosed at a late stage with concomitant poor prognosis (Duffy, 2001; Thomas and Sweep, 2001; Duffy, 2007). Although, tumor markers greatly improved 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 researchers 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. (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 is correlated with significantly shorter survival, and low expression of let-7a-2 has conferred poor prognosis in resected lung adenocarcinoma. Intriguingly, infour (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 showed that exosomes could be an important resource of cell-free miRNA in serum or plasma (Mitchell et al., 2008; Chen et al., 2008; Rabinowits et al., 2009). Chen et al. (2008) confirmed that serum miRNAs can serve as potential biomarkers for the detection of various cancers. Rabinowits et al. (2009) reported that there was different in total exosome and miRNA levels between lung cancer patients and controls, and similar between the circulating exosomal miRNA and the tumor-derived miRNA patterns.

In non-small-cell lung carcinoma (NSCLC), Chen et al. (2008) analyzed serum microRNAs from lung cancer. There were a total of 63 microRNAs in the serum of NSCLC patients that were not found in normal serum. Interestingly, they also found differences between the microRNA profiling found in the blood cells and serum of NSCLC patients, while there were no significant differences between blood cells and serum of healthy controls. They also demonstrated that miR-25 and miR-223, which have previously shown to be involved in the development of cancer, were over expressed in the serum of the patients with NSCLC and therefore may be used as molecular markers of NSCLC. Hu et al. (2010) found that 4 microRNA (miR-486, miR-30d, miR-1, and miR-499) signatures from the serum may serve as a noninvasive predictor for the OS of NSCL. Rabinowits et al. (2009) reported that microRNA signatures were not significantly different between circulating exosomal microRNA and the tumor-derived microRNA patterns by examining 4 paired lung cancer tissue and plasma samples.

Therefore, the author suggested that circulating exosomal miRNA might be useful as a screening test for lung adenocarcinoma. Furthermore, Rosell et al. (2009) suggested that the expression of specific circulating miRNAs was a good surrogate of tumor miRNA

expression, and initiated a new paradigm that would be useful not only for early diagnosis but also for prognostic and therapeutic decisions.

Circulating micro ribonucleic acids (miRNAs) in 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.

Several miRNAs that may serve as oncogenes have been found to be over expressed in liver tissues and cell lines. One of the most commonly identified up-regulated miRNAs in liver cancer was miR-21 (Resnick et al., 2009), which may promote oncogenesis by repressing expression of the tumor suppressor phosphate and tensin homolog (Piccart-Gebhart et al., 2005; Harris et al., 2007). 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. (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. (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. Qu et al. (2011) investigated whether measurement of serum levels of the miR-16, miR-195 and miR-199a, alone or in combination with conventional serum markers, can help to differentiated liver carcinoma from chronic liver diseases. They used sera from 105 liver patients, 107 chronic liver diseases patients, and 71 normal control subjects to measure the miRNAs expression by real-time PCR. They found that the addition of miR-16 to conventional serum markers improved sensitivity and specificity for liver cancer. However, the author thought that use of miR-16 for second-line testing in cases considered negative on the basis of conventional liver cancer markers should be explored in larger, prospective studies.

The limitation of using miRNAs to diagnose human cancer

Firstly, 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. (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. (2008) determined colon cancer tissue miRNA expression levels was by TaqMan RT-PCR. When the 26 upregulated miRNAs from the Schetter's study are compared to those from the Monzo study, only 14 miRNAs are in agreement (54%).

Secondly, Cancers treatment might affect the expression of circulating miRNAs. 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.

Thirdly, 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 analyzed 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 indicated that miR-21 was also up-regulated in leukaemic cancers.

At last, although these results demonstrated 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-characterized, family and population-based case-control and additional validation studies are warranted (Schwarzenbach et al., 2011; Wen et al., 2011).

Conclusion

Circulating micro RNAs in the patients may potentially provide a noninvasive strategy for predicting drug

response. Monitoring the genetic profile during treatment may enable doctors to better tailor therapy for individual patients. Serum or plasma micro RNA profiling may do so more accurately and effectively than an mRNA or protein abundance profile. This noninvasive approach is especially important because not all patients with lung or liver cancers have operable disease; therefore many of them do not have tumor tissue available for genetic analysis. In the near future, a controlled clinical trial is necessary to confirm these conclusions.

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