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Review Article

Circulating microRNAs as Cancer Early Diagnostic Biomarkers-Promises and Concerns

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Abstract

MicroRNAs (miRNAs) are a large family of small non-coding RNAs that negatively regulate protein-coding gene expression post-transcriptionally. Since the discovery of first miRNA in 1993 and the first reporting of the presence of circulating miRNA in 2008, numerous studies have demonstrated the potential involvement of miRNAs in almost all aspects of cancer. In aiming to identify minimally invasive cancer early diagnostic biomarkers, a large number of studies have shown differential expression profiles or signatures of circulating miRNAs between healthy control populations and various cancer patients. Unfortunately, the reported circulating miRNA signatures that have been claimed to be able to separate healthy controls and cancer patients are often sporadic and lack consensus among different studies. Apparently, further studies are needed to identify and overcome potential confounding factors that contribute to this poor reproducibility of reported circulating miRNA signatures before the findings could be translated into clinical practice. In this short review, we will briefly discuss the promises and concerns of circulating miRNAs as cancer early diagnostic biomarkers.

Keywords: Noncoding RNA; microRNA; Circulating microRNA; Cancer Diagnostic Biomarker

miRNA and cancer

The first microRNA (miRNA), lin-4, was discovered in 1993 and referred to as a "small regulatory RNA" [1]. The term "miRNA" was first introduced in 2001 when three groups simultaneously reported the findings of a big number of miRNAs in the same issue of Science [2-5]. Since then, many studies have shown that miRNAs are a large family of small non-coding RNAs (~22 nucleotide long) that negatively regulate protein-coding gene expression post-transcriptionally by interacting with messenger RNAs (mRNAs) [5,6]. The interaction of miRNAs with mRNAs occurs through imperfect base pairing between the miRNA and mRNA 3'-untranslated region (3'UTR), principally involving the seed region of 6-8 nucleotides at the 5' end of the miRNA, and causing mRNA degradation or translation inhibition (Figure 1). According to the latest version (June 2014) of miRBase [7], Release 21 of the database contains 28645 precursor miRNAs and 35828 mature miRNAs in 223 species. This recently updated miRNA database listed 1881 human precursors and 2588 human mature miRNAs. Many studies have shown that the expression levels of miRNAs are often altered in many human diseases and extensive studies in this field have focused on cancer.

The first paper showing that miRNA expression level was altered in cancer was published by Calin et al. in 2002 [8], one year after the term "miRNA" was introduced. Calin et

al. reported that two miRNAs (miR-15 and -16) located in a chromosome region deleted in more than 50% of B cell chronic lymphocytic leukemia (CLL) were found to be often deleted or decreased in CLL patients [8]. Since then, numerous studies have demonstrated that compared to the normal cells and tissues, miRNA expression levels have been changed in all kinds of tumor cells and tissues tested [9-11]. It is now well-accepted that abnormal miRNA expression plays crucial roles in cancer initiation and metastasis [12,13].

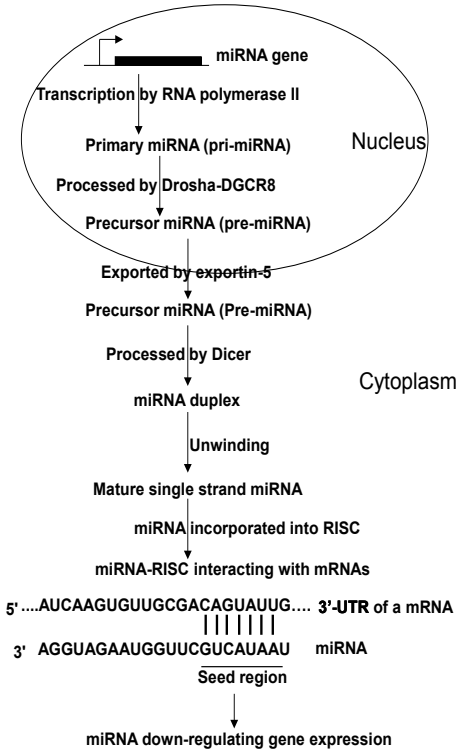

Figure 1. The biogenesis of miRNA and the mechanism of miRNA down-regulating gene expression. The miRNA genes are usually transcribed by RNA polymerase II to produce pri-miRNAs, which are processed by Drosha-DGCR8 (DiGeorge syndrome critical region gene 8) generating pre-miRNAs. These events occur in nucleus. The pre-miRNAs are exported to cytoplasm by exportin-5 undergoing the 2nd processing step performed by dicer producing miRNA duplexes. After unwinding the single strand mature miRNAs are produced, which are then associated with argonaute and incorporated into RISC (RNA-induced silencing complex). The miRNA-RISC interacts with 3'-UTRs (untranslated regions) of mRNAs and down-regulates protein-coding gene expression by causing mRNA degradation or translation inhibition.

Circulating miRNAs

Despite extensive studies, cancer remains to be one of leading causes of death in the United States and worldwide as well, which is mainly due to the fact that the majority of cancers are already at late stages upon diagnosis. Developing sensitive, reliable and minimally invasive biomarkers for cancer early diagnosis is thus one of the key strategies to reduce cancer mortality. Given the critical role of miRNAs implicated in cancer initiation and progression, circulating miRNAs have been actively explored as potential early diagnostic biomarkers for cancer.

The presence of miRNAs in plasma and sera was first reported by Chim et al. and Lawrie et al. in 2008 [14], respectively. Chim et al. found that the most abundant 4 placental miRNAs (miR-141, miR-149, miR-299-5p, and miR-135b) could be readily detected in maternal plasma during pregnancy and their levels were decreased in postdelivery plasma [14]. This is the first report showing that miRNAs exist in plasma and are exceptionally stable in cell-free plasma. Lawrie et al. reported almost at the same time that miRNAs were easily detectable in serum samples and that higher levels of specific miRNAs were associated with diagnosis and prognosis of diffuse large b-cell lymphoma [15]. This is the first paper showing the potential diagnostic and prognostic value of circulating miRNAs for cancer. Together, the findings from these first two pioneer studies show the presence of circulating miRNAs, suggesting that the circulating miRNAs may have the potential to serve as clinically useful biomarkers.

Circulating miRNAs as cancer early diagnostic biomarkers-promises

After the first two publications by Chim et al. and Lawrie et al. reporting the presence of circulating miRNAs, many subsequent studies have shown the differential expression levels of circulating miRNAs between healthy control populations and various cancer patients. Particularly, three research groups further characterized circulating miRNAs showing that miRNAs in sera and other body fluids are not associated with cells and are extremely stable even under various harsh conditions [16-18]. Interestingly, in striking contrast to highly stable endogenous plasma and serum miRNAs, it has been found that synthetic miRNAs added exogenously to plasma or serum samples were quickly degraded by the high level of ribonuclease (RNase) activity in the samples [16,18]. These findings indicate that endogenous circulating miRNAs are in some way protected from RNase degradation. Indeed, there is evidence showing that circulating miRNAs are packaged into exosomes [19,20] microvesicles [21] and apoptotic bodies [22], or associated with RNA-binding proteins [23] or lipoprotein complexes [24]. These findings provided novel explanation for the superior stability of circulating miRNAs, supporting the idea that circulating miRNAs may serve as potential biomarkers for cancer diagnosis.

In addition, technology advances in miRNA quantitative analysis have further fueled the studies on circulating miRNAs. While direct sequencing and miRNA microarray analysis are available, the majority of circulating miRNA studies has used

quantitative PCR (Q-PCR) assays, which are less technical demanding and cost effective.

Circulating miRNAs as cancer early diagnostic biomarkers-concerns

While a large number of studies have shown the differential expression levels of circulating miRNAs between control and cancer patients, however, concerns are arising about the clinical values of circulating miRNAs as cancer diagnostic biomarkers. One of the main concerns as revealed in recent review articles is the poor reproducibility of abnormally expressed circulating miRNAs for a given type of cancer reported by different studies [25-27]. After analyzing a total of 154 circulating miRNA expression profiles in 26 different tumor types in studies published from January 2008 to June 2013, Jarry et al. concluded that the circulating miRNA signatures claimed to be able to accurately separate healthy control and cancer patients lack concordance [25]. By reviewing 17 published studies describing dysregulated circulating miRNAs in gastric cancer patients, we and others found that while more than 30 miRNAs were reported to be significantly increased or decreased in gastric cancer patients' blood, serum or plasma samples, nevertheless, the reported changes of circulating miRNAs were mostly sporadic with very low consensus among different studies [26,27].

The lack of corroboration of reported circulating miRNA alterations in cancer patients from different studies could be attributed by multiple factors, which have been discussed in detail in recent reviews [25-27]. These may include (i) small patient sample sizes; (ii) different sample processing and analytic methods; and (iii) different normalization methods for quantifying circulating miRNA levels.

Summary

While a large number of studies have reported differential circulating miRNA expression profiles or signatures, the potential value of circulating miRNAs for serving as cancer early diagnostic biomarkers remains to be questionable. This is mainly due to lack of consensus of reported circulating miRNA signatures in a given type of cancer. Some key issues such as the origin of circulating miRNAs and methods for quantifying circulating miRNAs need to be better addressed before the findings on circulating miRNA studies could be translated into routine clinical practices for cancer early diagnosis.

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