Detection MicroRNAs as potential biomarkers non invasive and targeted therapy for several cancer

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ABSTRACT

MicroRNAs (MiRNAs) is small molecule non coding RNAs that consist of 18-25 nucleotide. It is important to control gene expression by affected post transcriptional and translational repression of mRNA. This RNA molecule may as oncogene miRNA (OncomiR) or tumor supresor gene (TSmiR). Lowest or enhanced of miRNA associated to increase development cancer cell, cardiovascular disease, metabolisme disease and immune disorders. The advantages of miRNA is about their stability in cell, body fluid that always stable under harsh condition as high temperature, different pH values, repeated freeze-thaw cycles and long-time storage. They are present not only in blood but also in formaline-fixed and paraffin embedded years before.The detection of miRNA in blood plasma and serum has potential for earlier cancer diagnostic and prognostic and response to therapy. In this review, providing researchers to increase understanding a glance of miRNA, biogenesis and their function.Better understanding may help to find spesifict biomarker for diagnosis and prognosis also to predict cancer targeted therapy.

Keywords: miRNA, diagnostic biomarker, prognostic biomarker, targeted therapy.

I. INTRODUCTION

MicroRNA was firstly discovered on 1993. One of regulatory miRNA in lin-4, called small temporal RNA, was found in Caenorhabditiselegans (1).Until now, affect from this molecule is still mysterious. Several studies showed that unique in plant miRNAs, frequently have pair one or a few targets, also it's interactions reliably have important role to their key functions (2). MicroRNA are small non coding RNA that have 22 nucleotide long bp regulatory, derived from endogenous short hairpin transcripts, have important role in development and physiological processes in most organism (3). Different classes of ncRNA act regulator celuller processes and many of them have associated with cancer heterogenicity. It's bind to 3'UTR of target RNA, resulting in its degradation or translational inhibition. Deregulation of miRNA occurs in cancer and may as oncogenes or tumor suppressor genes (4).

1.1. Biogenesis of miRNAs pathway

MicroRNA have similary characteristic like the other RNA. Firstly from all processes start from nucleus. This molecule have account approximately 1% of the human genome and are highly conserved in nearly all organisms (5). In mammals are predicted to regulate more than 50% of all protein coding genes (6). They act inhibition translational by binding to complementary sequences in the 3'untranslated region (UTR) of mRNA targets. For this function, protein expression will be repressed either by inhibition of translation or by degradation of the mRNA targets. To bind for their targets, a sequence of only 2–7 nucleotides is needed. In most cases, miRNAs bind only in part to their complementary target mRNA sequence. If the incomplementary strand binding leads to repression of translation or deadenylation of the target mRNA, a complete complementary binding leads to degradation of the target. In this process, miRNA binds to two proteins (GW182 protein and any of the proteins of the Argonaute [AGO] family) and forms a miRNA-induced silencing complex (miRISC) (Figure1). Its molecule then serves as the inhibitor of translation (7). MiRNA expression is controlled by transcriptional regulation. Two kinds of ribonuclease III (RNase III) type endonucleases—DROSHA and DICER1—and their cofactors are involved in the cleavage of precursor miRNA, and the efficiency processing depends on binding to a variety of molecules, including transcriptional factors (such as p53 and NANOG), receptor proteins (such as α-ER and EGFR), and signal transducers (such as SMAD, MAPK and ERK), this factor can increase or decrease activity of transcription of miRNA (8). MiRNA are transcribed by RNA polymerase II (RNA Pol II) from either clustered miRNA genomic loci of several kilobases (kb), or co-transcriptionally from introns of other genes, generating hairpin-structured primary precursor transcription units (pri-miRNAs) (9). In this cases pri-miRNA arecropped at the hairpin stem to 70 bp(pre-miRNAs) by the nuclear RNase III-type protein Droshaadded by the DiGeorge syndrome critical region gene 8 protein(DGCR8) (10). Pre-miRNas was transcripted in nucleus. Then, this molecule are exported to the cytoplasm by the nuclear transport receptor Exportin5 (EXP5, XPO5) and cleaved close to the terminal loop bythe RNase III-type nuclease Dicer and releasing 22 bpdoublestranded RNA duplex(11). One of miRNAs strand to be active miRNA or guide major strand and will be loaded to an AGO protein to be the miRISC complex, but the other strand will be degraded (9).

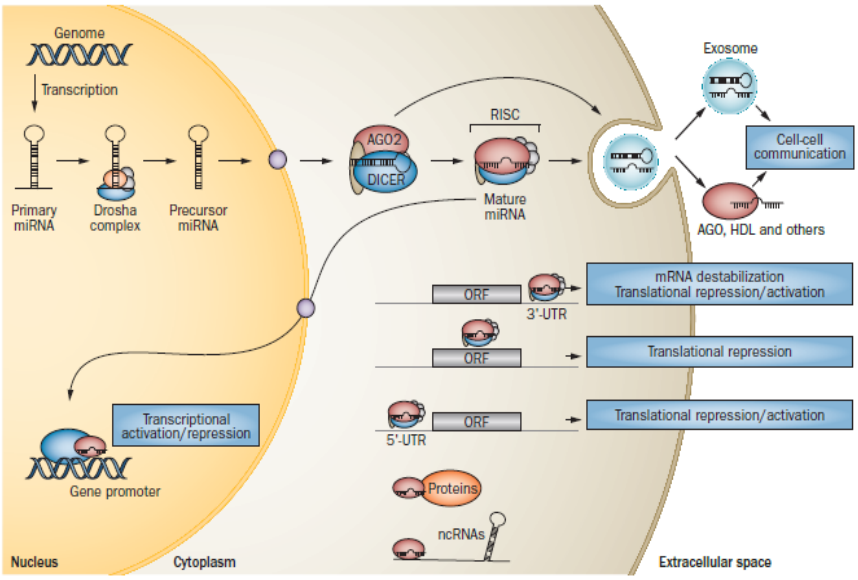

Figure 1. Biogenesis of miRNA (Schwarzenbach, H. *et al*., 2014)

In addition, some author showed that the complexmechanisms regulating microRNA function on target mRNAs. MicroRNAs recognize complementary sequences inthe 3´ UTRs of their target mRNAs and can also bind to the 5´ UTR or the open readingframe (12).Surprisingly, they can upregulatetranslation upon growth arrest conditions (13) (Figure 2).In addition, mature miRNAshave specific hexanucleotide (AGUGUU)sequence which acts as a transferable nuclear localization element (14). In other hand, miRNA can be secreted by vesicle on the cell, known as

exosomes, may contain both mRNA and microRNAs, can be delivered to another cell and be functional in this newlocation. These RNA molecules represents a novel mechanism of genetic exchange (15).

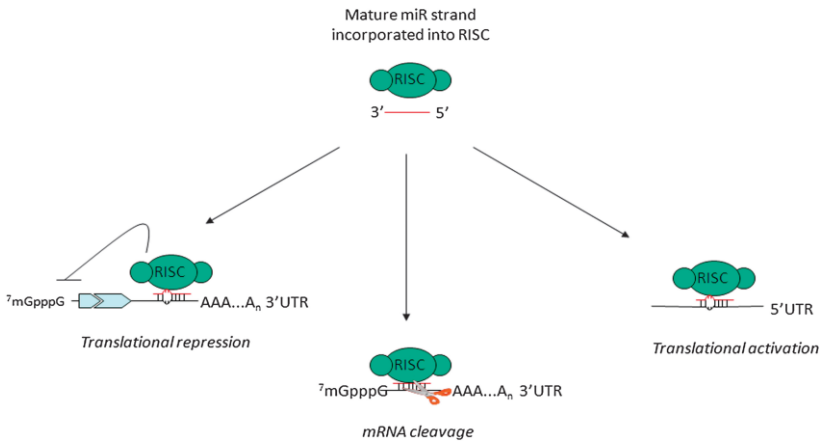

Figure 2.microRNA function on target molecules.

1.2. Technology available detection of miRNA

Alteration of miRNA have associated and related to cancer. This molecule can act as oncogene or tumor suppressor. Some study showed that blood-based protein biomarkers, such as carcinoembryonic antigen (CEA), carbohydrate antigen (CA), or prostate specific antigen (PSA), have gained a lot of recognition and can indicate abnormality condition (16). MicroRNAs have potential to bean essential and indispensable layer in gene regulation, mainly,post-transcriptional regulation. Involved in many physiological and can as biomarker.

miRNAscan find in the cell-freebody fluids such as plasma, serum, urine, saliva, usually termed ascirculating miRNAs, have been exploited as biomarkers in manydiseases in the past five years. Placental miRNAs were the firstclass of miRNAs to be detected in maternal plasma
during pregnancy (17). In other hand, this molecule were found to be elevated in the serum of lymphomapatients compared to healthy individuals (18).Then circulating miRNAs have found always in circulation and was detected for various diseases, features such easy of access and remarkable stability

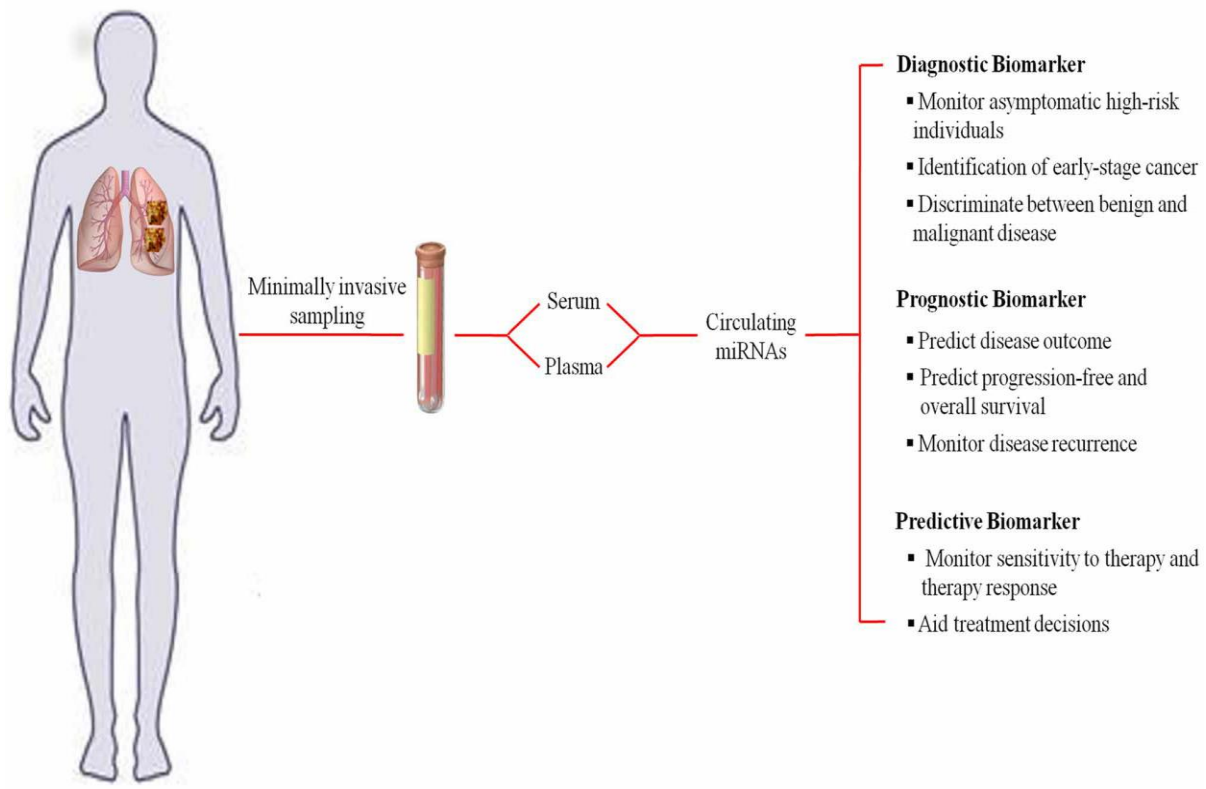

Figure 3. predictive uses of circulating miRNAs as a biomarker using lung cancer as an example (Madhavan*et al.*, 2013).

MicroRNA expression always have alteration for several cancer. Genome-wide expression-profiling studies using technologies have used that almost all cancer types present a specific profile of upregulatedor downregulatedmiRNAs (19). miR-125b,which was reported have function as either an oncogene or tumor-suppressor gene in different cancer types or cell lines (Figure 4). In ovarian, thyroid, and oral squamous-cell carcinomas, *miR-125b* is downregulated and has been shown to inhibit cell proliferation and cell-cycle progression (20).

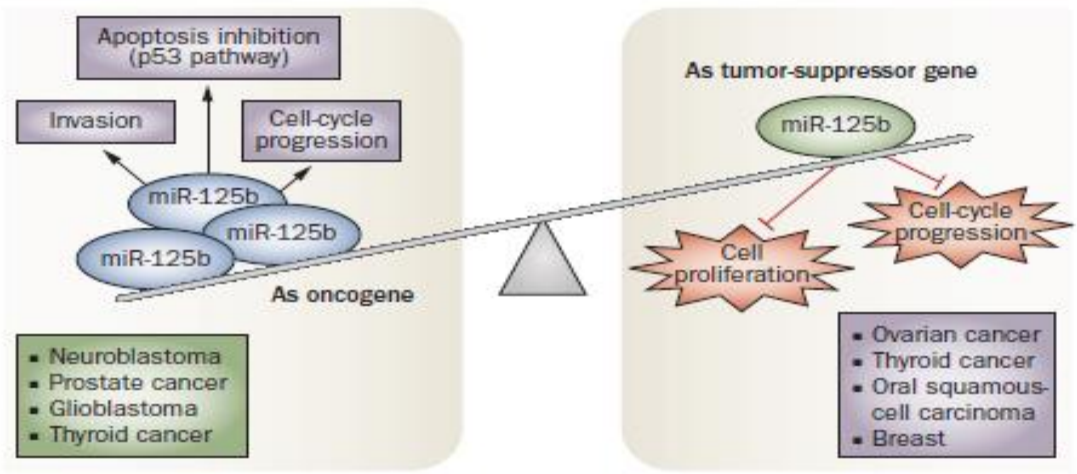

Figure 4. A miRNAfunction as both an oncogene and tumor-suppressor (Cortez, M. A. *et al,* 2011).

Upregulation of miR-21expression levels were associated with poor survival and therapeutic outcome in 84 patients with colon adenocarcinoma (21). In other research, showed that cancer patients had significantly higher salivary levels of *miR-31* than the controls, and eight of nine patients had a decrease in salivary *miR-31* levels after tumor resection. These results were also found for *miR-31* plasma levels. Increased levels of *miR-126, miR-152,* and *miR-182* were found in urine samples from patients with bladder cancer, and the ratios of *miR-126* to *miR-152* and *miR-182* to *miR-152* indicated the presence of bladder cancer with a specificity of 82% and a sensitivity of 72% (22). Another study in NSCLC reported that a set of 11 serum miRNAs were differentially expressed between patients with longer or shorter survival, and among this set, four (*miR-486, miR-30d, miR-1,* and *miR-499*) were associated with decreased overall survival of patients (23). Some research showed that miR-145 was reportedto be upregulated in serum of PBC (24). Recently, analysing circulationmiRNAs that are known to be aberrantly expressed in breasttissue or have functional role in tumorigenesis has led to theaddition of miR-21 and miR-92a (25), miR-10b, miR-125b, miR-155, miR-191, andmiR-382 (26)and miR-30a (27) to this growing list of miRNAsfor early detection of PBC. Deregulation of miRNA can easy detect with several technology.

Several techniques are currently available for detectmiRNA signatures in body fluids, such as miRNA microarrays,quantitative real-time PCR (qRT-PCR),and deep sequencing (next-generation sequencing).The most frequently used is qRT-PCR and its variations, such

as stem-loop RT-PCRand poly(A)-tailed RT-PCR,which have improved the specificity and sensitivity of miRNA detection. We are still to explore more consortium approach research to development potential biomarker on cancer.

Technique	Advantages	Limitations
RT-PCR	High sensitivity and specificity High dynamic range Suitable for quantification No need for special equipment User and lab friendly	Low/medium throughput Can detect only annotated miRNAs
Microarray	High-throughput Relatively low cost	Not suitable for accurate quantification Can detect only annotated miRNAs Low dynamic range
Deep sequencing	Detection of novel miRNA Detection of splicing variants (isomiRs) Ability to distinguish similar sequences	Sequence-specific bias High cost Need for special equipment and bioinformatician Relatively high amount of starting material

Figure 5.Main technologies to quantify miRNAs in body fluids(Schwarzenbach, H. *et al.*, 2014)

New method can be useful to increase information to support clinical threatment. The collaboration all of expert is very important to treat cancer patient in the future. So, with available method now that our change for increase our know ledge and skill on medical research.

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