

# Circulating MicroRNAs as Biomarkers in Health and Disease

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## ABSTRACT

In the recent years, circulating nucleic acids have emerged as new biomarkers. Among these, microRNAs(miRNA) have evolved as promising and potential markers of both physiological and pathological conditions. MiRNA are transcribed from DNA like the other mRNA molecules. Their secretions and functions have to be still explored in humans, though many theories have been proposed. It is a small non coding RNA which

plays an important role in the regulation of the gene expression, cell-cell communication, cell division and apoptosis. MiRNAs are stable and tissue specific and they can be identified and quantitated, which make them ideal biomarkers. This review highlights the secretion, mechanism of action and the role of miRNA in the diagnosis and the management of different disease conditions.

**Key Words:** miRNA, Biomarkers, Expression, PCR and microarray

## INTRODUCTION

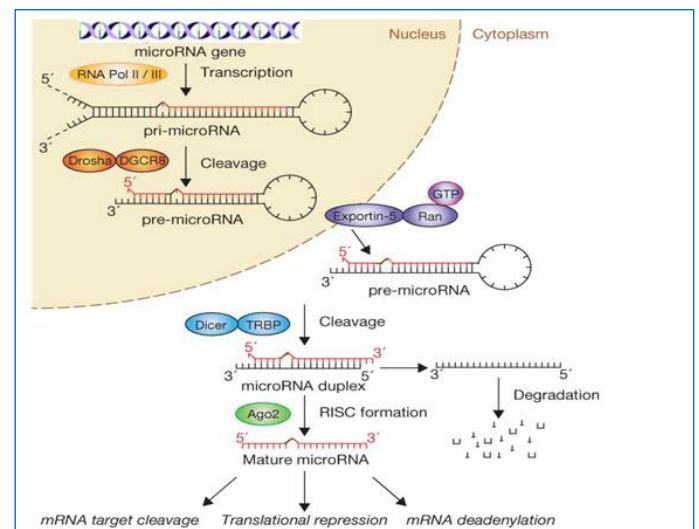
Sera and other body fluids contain cell free DNA, RNA and circulating nucleic acids which serve as potential biomarkers [1]. One such biomarker which has aroused interest in recent years is a small non-coding (18 – 24 ~nt) RNA which is called as microRNA (miRNA) [2]. It was discovered in 1993 by Ambros et al., in *Caenorhabditis elegans* [3]. The important roles of miRNA are regulation of the gene expression, cell-cell communication, cell division and apoptosis [2]. Recent data show that there are over 1500 matured and primary miRNA(pri miRNA) which are found in humans, with certain definite functions.

The MiRNAs are transcribed similarly as protein coding genes by RNA polymerase II [4] and RNA polymerase III [5]. The pri-miRNA is transcribed in the nucleus and undergoes post transcriptional processing in the cytoplasm, which is similar to that of other RNAs. DROSHA and DICER are RNase III enzymes which are involved in both the phases. During its processing, the ~70nt pri-miRNA is converted into 18 – 24 ~nts matured double strand miRNA (ds miRNA) [Table/Fig-1 & 2] [6].

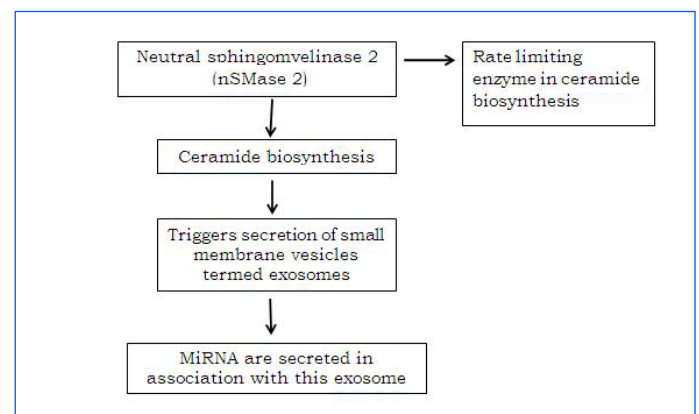
One of the functions of miRNA is downregulation of the gene expression, which makes it a useful diagnostic and prognostic tool in cancer [7]. It has high stability in blood, which enables an easy storage of the sample for the estimation of miRNA [8]. The current studies have provided a new algorithm for the screening and diagnosis of various diseases by using miRNA. This review pertains to the whole process of biogenesis, secretion, regulation and the use of miRNA in health and diseases.

## THE CURRENT STATUS OF MIRNA AS A BIOMARKER

Biomarkers may be defined as biological substances which are more specific and sensitive to a particular physiological or a pathological condition [Table/Fig-3]. The present studies show that miRNA fits into all the criteria for biomarkers [Table/Fig-4]. It is stable in various body fluids; the sequences of most of the miRNAs are conserved among different species; the expressions of



[Table/Fig-1]: Biogenesis of Pathway of miRNA



[Table/Fig-2]: The secretion of miRNA

some miRNAs are tissue specific and the biological conditions as well as the levels of miRNAs can be assessed by using modern methods. Recently, we use specific proteins, enzymes and isoenzymes as biomarkers for the diagnosis of many a disease.

Let -7 – miRNAs family	Stabilizes the self-renewing versus differentiated cell fate
Bantam miRNA	Physically associated with dFmrp in the ovary. It acts as an extrinsic factor for germline stem cell maintenance. Bantam interacts genetically with dFmr1 to regulate the fate of germ line stem cells
miRNA – 223	Plays an important role in differentiation and function of osteoclasts. Decreased osteoclast number and bone resorption. It regulates NF1 – A and H – CSFn levels
miRNA- 128	Overexpression in glioma cells inhibits cell proliferation. Target site is within the 3'UTR of E2F3a, a transcription factor which regulates cell cycle progression. The protein level of E2F3a negatively correlates with miR-128

**[Table/Fig-3]:** MiRNAs in cell differentiation and development

Specific	Specific tissue.
Sensitive	Can be differentiated in all pathological conditions. Early and significant variation in levels during the different stages of disorders.
Predictive	Long half-life.
Robust	Easily identified and quantified using modern techniques.
Translatable	Data can be used to bridge pre-clinical and clinical gap
Non invasive	Present in circulation.

**[Table/Fig-4]:** Characteristics of an ideal biomarker

The examples include troponin in the diagnosis of myocardial infarction, Prostate Specific Antigen (PSA) for prostate cancer and Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) enzymes for the liver function, etc [6].

The serum miRNA studies began with the associated levels of miR-21 in patients with diffuse large B-cell lymphoma. The expression of the miRNA profiles for lung cancer [Table/Fig-5], colorectal cancer, Diabetes mellitus and other diseases has also assumed relevance. Any biological sample can be used to assay the miRNA. Presently, the circulating miRNAs are easy to detect and to quantitate.

In prostate cancer, the level of miR-142 is significant, as compared to that in healthy individuals [9]. In urine, the miR-126 and the miR-182 ratio helps in the diagnosis of bladder cancer [10]. In saliva, low levels of miR-125a and -200a detect oral squamous cell carcinoma [11].

## MIRNAS AND CANCER

Previously, carcinogenesis was attributed to abnormalities in the oncogenes and the tumour-suppressing genes. Recently, it was recognized that miRNA also possesses an important role in the diagnosis and prognosis of cancer. The links between the dysregulation and the reduced expression of the tumour suppressor genes in cancer were shown in earlier studies [12]. Calin et al., located miR-15 and miR-16 on chromosome 13q14, a region that is deleted in more than half of the patients of chronic lymphocytic leukaemia and B-cell leukaemia [13]. The overexpression of the let 7 family on the RAS oncogenes lead to lung cancer [14]. MiRNAs are also called 'oncomirs', because both in in vitro and in vivo conditions, they can be viewed during the cell growth, cell cycle progression and during the invasion in all types of cancers [15]. A series of studies (He et al., Lu et al.,; O'Donnell et al.,) has reported the association of miRNAs and

miR-15 & miR-16	Absent or downregulation in chronic lymphocytic leukemia
miR-34a	Absence of miR-34a is seen in pancreatic cancer cell, induced by p53 tumor suppressor protein. Involved in cell cycle progression, apoptosis, DNA repair and angiogenesis. Its expression is silenced and attributed to aberrant CPG methylation of its promoter.
miR-23b	Repressed in Acute Myelogenous leukemia (AML)
miR-155	Increased expression in AML

**[Table/Fig-5]:** MiRNA expression and tumor genesis

Condition	MiRNAs
Diffuse large B-cell lymphoma	Increased levels of miR-155, miR-210 and miR-21
Prostate cancer	Distinguishing level of miR-144
Breast cancer	Increased levels of miR-10b and 34a Decreased levels of miR-195, let-7a
Ovarian cancer	Increased levels of miR-21, -141, -200a, 200b, 200c, 203 205 and miR-214
Colorectal cancer	Increased levels of miR-29a, -17-3p and miR-92a (Plasma)
Bladder cancer	Ratio of urinary miR-126 and miR-182
Oral cancer	Increased levels of miR-31 (Plasma) and miR-125a, -200a (saliva)
Myocardial Infarction	Increased levels of miR-499 and miR-208b
Congestive Heart Failure	Increased levels of miR-423-5p
Coronary Artery Disease	Decreased levels of miR-126, cluster miR-17/92 and miR-155a

**[Table/Fig-6]:** miRNAs putative biomarker in different pathological conditions

cancer [16–18]. The dysregulation of a group of miRNAs resulted in overexpression of the MYC oncogenes, which is associated with B-cell neoplasia [12].

A study which was performed by Arndt et al., showed a significantly low level of expression of miR-143 and miR-145 in colorectal carcinoma [19]. Similarly, miR-10b, miR-125b and -145 are downregulated and miR-21 and miR-155 are upregulated in breast cancer [20]. Among the miRNAs, miR-145 is involved in downregulation. Moreover, it helps in finding out the progression of the normal breast tissues into cancerous tissues. The Mi-21 expression is progressively upregulated during the progression of breast cancer. Some of them are associated with the invasion and the prognosis of breast cancer [21]. Tumours are classified, based on their miRNA expression, which can be used in their diagnosis and prognosis [Table/Fig-6].

## MIRNAS IN THE NERVOUS SYSTEM (NS) REGULATION

MiRNAs play an important role in maintaining the survival of the mature neurons and their functions. MiRNA-134 contributes to the synaptic development, maturation and plasticity. A defective expression of miR-134 could be associated with diseases viz., Alzheimer's disease, the Fragile-X-Syndrome (FXS) and autism [22]. MiR-133b regulates the maturation and the function of the midbrain dopaminergic neurons and it is deficient in patients with Parkinson's disease [23]. MiR-8 directly targets atrophin and the miR-8 mutant phenotypes are attributable to the enhancement of

the datrophin activity, resulting in elevated apoptosis in the brain and behavioural defects. MiR-124 promotes the NS development by regulating an intricate network of NS specific alternative splicing. Mi-124 also affects certain regulators of the neuro-specific gene expression [24].

MiRNA-124 directly inhibits the small C-terminal domain phosphatase-1 (SCP-1) which is required for the RE1 silencing transcription factor (REST) mediated repression of the neuronal genes [25]. When cells differentiate to neurons, the REST transcription is inhibited and miR-124 promotes the elimination of the biological effects of REST by inhibiting SCP1 [26].

## MIRNAS AND VIRAL INFECTIONS

The host cells use miRNAs to target certain essential viral functions. In turn, the viruses use miRNAs to control their host cells. An anti-viral miRNA against the retrovirus primate foamy virus type I (PFV – I) in human cells was the first to be reported [27]. The SV 40 encoded miRNA -miR-S1 helps in keeping the infected cell hidden from the immune system. It is expressed late in the viral replication cycle and it helps in degrading the viral mRNA encoding T antigen. This limits the exposure of the infected cell to the cytotoxic 'T' lymphocytes [28]. The expression of the host cell miR-122 can inhibit the replication of the hepatitis C virus and it works through IFN –  $\beta$ . The miRNA silencing machinery plays a physiological role in controlling the HIV – 1 replication [29].

## MIRNAS AND THE CARDIOVASCULAR SYSTEM

Coronary heart disease is still the leading cause of death worldwide. Recent studies have indicated that several miRNAs are dysregulated in CAD patients. In cardiovascular diseases, several specific circulating miRNAs have been profiled as novel biomarkers for distinguishing among the different cardiovascular events. Studies which had been performed in early 2008 have demonstrated the link between the circulating miRNAs and diseases other than cancer [8,9,30].

Adachi et al reported that miR-499 was elevated in acute myocardial infarction, but that it was not elevated in coronary heart disease [31]. Wang et al presented miR-208a as a sensitive, early marker of AMI, because the levels got elevated within 4 hrs [32]. Corsten et al., observed significant correlations concerning the levels of miR-208b and miR-499 with the serum levels of troponin T and Creatine Kinase (CK), which are two established markers of cardiac injuries [33].

Tijssen et al., studied 108 miRNAs by using microarray and reported that they were differentially expressed in coronary heart failure patients. A further analysis which was performed by RT-qPCR showed a rise in miR-423-5p in other Congestive Heart Failure (CHF) patients. It had a correlation with the N-terminal pro-hormone brain natriuretic peptide levels (NT-proBNP) and the ejection fraction, suggesting that it would be a good predictor of the CHF diagnosis [34].

Fichtlscherer et al., in the plasma of CAD patients, detected low levels of the miRNAs, namely miR-126, the miR17/92 cluster and miR-208b and also found them in low levels at the baseline. A prospective evaluation of the data showed low levels of miR -126, -17, 92a and miR-155. The MiRNAs which were packed in exosomes, microvesicles or apoptotic bodies, which were taken up by

atherosclerotic lesions, led to a speculated reduction in the levels of the circulating miRNAs in CAD patients [35].

## MIRNAS AND GASTROINTESTINAL DISEASES

Recently, it was identified that 22 miRNAs were regulated and that 13 were downregulated in gastric cancers [36]. MiR-15b and miR-16 were down regulated in human gastric cancer cells [37]. They act by modulating apoptosis. A considerable upregulation of miR-135a and miR-135b was observed in colorectal adenomas and carcinomas and it was correlated significantly with the low Adenomatous Polyposis Coli(APC) mRNA levels. MiR-135a and miR-136b target the 3'UTR of the APC gene and cause its suppression. Thus, microRNAs are very much associated with the pathogenesis of colorectal cancers [38].

## MIRNAS AND DIABETES MELLITUS

MiR-7 and miR-375 are expressed in high levels in the pancreas. They are associated with both the development of the pancreas and also the secretion of insulin. MiR-375 directly targets 3' phosphoinositide- Dependent Protein Kinase – 1 (PDK-1) and it decreases the glucose stimulatory action on the insulin gene expression [39]. Glucose decreases the miR-375 precursor level and a concomitant increase in the PDK-I protein [40]. The biological mechanism of a recently discovered association of type 2 diabetes with an ACAA insertion/deletion polymorphism at 3' UTR of the IGF2R gene can be very well explained on the basis of the role of miRNA [41]. Decreased levels of miR-20b, miR-21, miR-24, miR-15a, miR-126, miR-191, miR-197, miR-223, miR-320 and miR-486 were significant in diabetes, but there could be a moderate increase in miR-28-3p [35].

## THE ANALYSIS OF MIRNA

Recent studies have depicted that miRNAs could be used as non-invasive markers for the diagnosis and prognosis of conditions like acute myocardial infarction, congestive heart failure, cancer and drug induced liver damage [32,34,42]. miRNAs showed resistance towards their degradation by RNase, which made them a promising diagnostic tool as compared to the miRNAs [8,9,43,44].

Only, few data are available which pertain to the analytical assays of miRNAs. Jennifer et al discussed the pre-analytical and the analytical errors of the circulating miRNAs. The concentrations of the miRNAs will be altered by pre-analytical factors like the protocol of the sample collection, centrifugation (rpm) and stability. There will be an increase in the total miRNA concentration due to contamination of the sample with platelets, erythrocytes and haemolysis. Usually, miRNA is stable for 24hrs at room temperature and for 72hrs when it stored at – 4°C [45].

The quantification of miRNA is performed both in plasma and serum. Both the serum and the plasma concentrations of the miRNAs are different. As compared to plasma, serum depicts a good correlation as a marker, even though serum contains a low concentration of miRNA. Commercial kits are available for the extraction of miRNA from other body fluids [46].

A spectrophotometer rarely determines small amounts of miRNA. Alternative modern techniques such as Real Time PCR (RT-PCR) for qualitative identification and qPCR can estimate its concentration by measuring the electrical signals which are formed from the oxidation of guanine which is induced by the hybrid formation of



miRNA [47]. The microarray technique provides functional genomics through the parallel expression measurements of the genomes, which could be used in the research which pertains to drug discovery and the targeting and the determination of the biomarkers. Microarray enables the quantitation and the sequencing of miRNA and this could ultimately be acceded into the database [48].

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