*Review Article*

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# A Recent Update on the Extracellular Micro RNA as Novel Biomarkers in the Diagnosis of Human Cancers

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**Abstract**

Most of the available non invasive biomarkers for the detection of human cancers are reliable in the metastatic disease or able to monitor the primary/ adjuvant chemotherapy for the relapse. Hence, an ideal biomarker especially to detect cancer at an early stage is urgently required to reduce the morbidity and mortality worldwide. microRNAs (miRNA or miR), small non-coding RNAs (18-25 nts) synthesized from the human genome were found to be involved in a variety of cellular processes such as cell proliferation, differentiation and programmed cell death. A unique tissue miRNA expression profile was found in the malignancy of breast, bladder, colon, lung, liver, brain, prostate and pancreas, had proposed their role in the diagnosis of cancer as well as the treatment prognosis. The altered expression was also associated to the metastasis of the cancer cells as well. Among the various methods used for their detection in biological fluid such as blood, urine and saliva, quantitative reverse transcriptase polymerase chain reaction is the most commonly employed technique. Though increasing evidences had suggested the importance of miRNAs, a quality assisted standardized procedure for their detection is fragmentary. Therefore, standardization are urgently needed mainly in the processing of sample, developing a more accurate and precision method along with the normal extracellular housekeeping RNAs which can be used for the normalization to assess the quality and quantity of miRNA. This review article discusses recent update on the role of circulating extracellular miRNA in the diagnosis of human malignancy.

**Keywords**: Micro RNA; Gene Silencing; Tumor Suppressor Genes; Oncogenes; RNA Interference; Quantitative Reverse Transcriptase Polymerase Chain Reaction; Chronic Lymphocytic Leukemia.

## Introduction

The silencing of gene at the post transcriptional level by RNA interference (RNAi) can regulate the expression of certain genes whose products are involved in the tumor initiation. RNAi silences genes by modulating their action through exploiting the Watson-Crick base pairing with the target mRNA molecules and finally promote the degradation of RNA in the cytoplasm. The mechanism of RNAi can be mediated either by the use of small interfering RNA or by the naturally occurring cellular microRNA (miRNA or miR). miRNAs were initially discovered in 1993 [1]. Since then, more and more evidences suggested their role in various biological processes of human cells [1,2]. The complex molecular mechanisms underlying the carcinogenesis include the genomic instability and expression of abnormal protein products that generate continuous growth signals to derange the normal cell cycle process. Cancer can be viewed fundamentally as a genetic disease where two sets of genes- cellular oncogenes and tumor suppressor genes- were altered. While a mutant tumor suppressor's alleles are usually recessive, the mutant oncogene alleles are typically dominant.

Altered expressions of several mi RNAs were described in the early and advanced stages of human cancers. They are shown to be stably expressed in serum, plasma and other body fluids. The advances of cancer diagnosis lie in the use of biomarkers (tumor markers) that offer the potential to identify and treat cancer years before it is either visible or symptomatic. The tumor markers that are being used currently in

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the clinical practice will fall into one of the several categories (Figure 1). However, most of them are useful either in the advanced stages of metastatic spread or to detect the relapse from the therapy. An ideal tumor marker should fulfill certain criteria that range from the early detection to the rapid, simple, accurate and inexpensive method of detection. Advances in imaging technologies open up a new era for the detection of novel biomarkers. This review article discusses the recent update in the role of circulating extracellular miRNA as novel biomarkers in the diagnosis of cancer in human.

*Synthesis and Secretion of MicroRNAs*

MiRNAs are small non-coding RNAs (18-25 nts) to regulate a large variety of cellular processes such as cell proliferation, differentiation, programmed cell death, immunity, metabolism and stem cell maintenance [3]. Human genome consists of more than 1000 genes (1- 5% of the total genes) for synthesizing miRNA and about 800–1000 miRNAs were reported in human. Cellular gene expression can be regulated at the post transcriptional level by miRNA. The physiological and therapeutic roles of miRNA in cancer had been previously reviewed by Ajith [4]. The synthesis and processing of miRNA is given in figure 2. They are transcribed mainly by RNA pol II as long primary (pri)-miRNAs in the nucleus, subjected to Drosha- a class 2 ribonuclease III enzyme-action to form pre-miRNA of ~70 nts. They are exported to cytoplasm where they are further subjected to Dicer- RNase III enzyme- to form 18-25 nts long double stranded RNA. After processing in the cytoplasm, one of the two strands of mature miRNA will be incorporated in the ribonucleoprotein complex called miRNA containing RNA induced silencing complex (RISC) and the other strand degraded in the cytoplasm. The Argonaute proteins, an endonuclease of the RISC complex will direct the

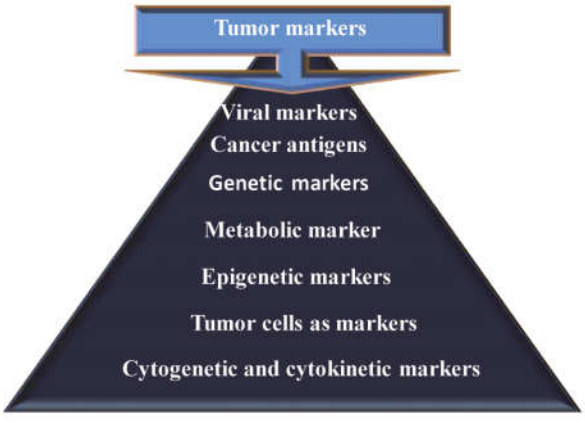


**Fig. 1:** Categories of biomarkers used in the human cancer

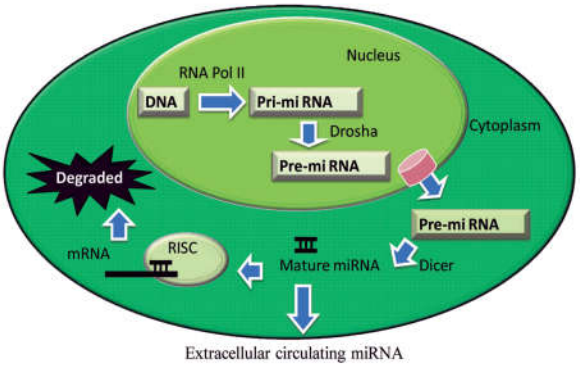


**Fig. 2:** Formation, maturation and secretion of microRNA (miRNA). miRNA is formed as pri-RNA by RNA pol II. They are processed in nucleus and later in cytoplasm by RNase III, Drosha and Dicer, respectively. One of the stands of mature miRNA incorporated in RISC which base paired with mRNA resulted in the post transcriptional silencing. miRNA secreted extracellularlly RISC: RNA-induced silencing complex; RNA pol II: RNA polymerase II

Watson-Crick base pairing between the bound miRNA and the 3′ or 5′ untranslated regions (UTR) of the targeted mRNA leading to degradation and thus the post transcriptional silencing of the mRNA. Further, mature miRNA will be secreted extracellularlly after incorporated either in the lipid vesicles or packaged within the protective exosomes and kept away from the degradation by RNase in the extracellular fluid such as blood, urine and saliva.

*Role of MiRNA in cancer*

Over the past decade, experimental evidences showed that the miRNAs as a new paradigm of gene regulation in cancer. They are involved in a wide array of carcinogenic processes where they can be up- or down-regulated. Over-expressed miRNAs may function as oncogenes by down regulating the tumor-suppressor genes, whereas miRNAs that act as tumor-suppressor genes are down regulated. The first evidence of the involvement of miRNAs in human cancer was derived from the studies on chronic lymphocytic leukemia (CLL). Calin et al [5] reported that a deletion of chromosome 13q14 in approximately 69% of CLLs lead to the loss of two miRNAs, miR-15a and miR-16-1.

The interaction of miRNA with the regulators of cell cycle such as Cyclin-CDK complexes or cell cycle inhibitors of the INK4 families ($p16^{INK4a}$) or Cip/ Kip ($p21^{Cip1}$ or $p27^{Kip1}$) had been reported [6,7]. At least nine members of mammalian let-7 (let-7ai) family of miRNAs negatively regulated *RAS* proto-oncogenes, *H-RAS, K-RAS* and *N-RAS* through multiple complementary sites in the 3′-UTRs of all three human *RAS* mRNAs [8]. The deregulated miRNA expression

in cancer can be either due to multiple mutations or from the epigenetic changes such as altered DNA methylation [9]. Since, a unique tissue miRNA expression profile was found, the expression of miRNA allowed different types of cancer to be discriminated with high accuracy which may help to identify the tissue origin of poorly differentiated tumors [10,11].

*Altered Expression of miRNA in Human Malignancies*

miRNA can either be increased or decreased in level during the malignant transformation of cells. Hence, their level is altered in the biological fluid. Review literature during the past decade had revealed the altered expression of miRNA in malignancies such as neoroblastoma, glioma, melanoma, renal cell carcinoma, cancer of breast, prostate and pancreas.

*Neuroblastoma and Glioma*

Quantitative-RT-PCR miRNA profiling on RNA from 53 serum samples, representing 33 diagnostic cases of common childhood cancers plus 20 controls found that a panel of miRNAs such as miR-124-3p/miR-9-3p/miR-218-5p/miR-490-5p/miR-1538 have potential role in the clinical management of neuroblastoma. They are consistently over-expressed in *N-MYC* amplified high-risk cases [12]. miR-125b could be a potential biomarker with relatively high accuracy in the diagnosis of glioma. Serum miR-125b level was found significantly lower in glioma patients when compared with the normal population. Furthermore, decreasing trend of miR-125b level along the tumor stages was found [13]. The sensitivity, specificity, and AUC for miR-125b in human cancers diagnosis were 82 %, 77 % and 0.84, respectively.

*Renal Carcinoma*

Expression of miR501-5p found to be a possible biomarker for the prognosis of clear cell renal carcinoma, which has reported to be poor prognosis. Since, miR501-5p up regulation can enhance the activity of mammalian target of rapamycin and promote both cell proliferation and survival, the down regulation of miR501-5p can be considered as a promoter of good prognosis [14]. Li et al. [15] suggested miR 130b as a possible novel biomarker.

*Breast Cancer*

MiR-21 has a relatively high diagnostic value for detecting breast cancer. miR-21 assays in plasma, serum and also in tissue achieved relatively higher accuracy [16]. Du Rieu et al. [17] demonstrated that over expression of miR-205 and miR-21 as new potential biomarker in the early diagnosis of ductal adenocarcinoma. miRNAs of miR-200 family were differentially expressed between basal and luminal breast cancer subtypes and can specifically classify estrogen receptor (ER), progesterone receptor (PR) and HER2/neu receptor status [18-22]. miR-145 and miR-205, preferentially expressed in normal myoepithelial cells, were dramatically reduced in basal-like triple negative tumours (ie. ER-/PR-/HER2-), suggested that their expression change might be a consequence of disease progression [19]. The expression of miR-7 was decreased in metastatic breast cancer, correlated with the level of epithelial differentiation of the tumor and such expression was found to inhibit the metastatic progression [23]. miR-300 was down-regulated in the breast cancer cells that underwent epithelial-to-mesenchymal transition [24]. Furthermore, high levels of miR-125b and miR-21 predicted poor response of patients to treatments in breast cancer and pancreatic cancer, respectively [25,26]. Over expression of miR-221 and miR-222 was associated with the resistance to tamoxifen therapy [27,28]. The expression levels of micro RNAs such as miR-146a-5p which is up-regulated and miR-181d and miR-195-5p which are down-regulated in HER2-positive breast cancer and can significantly predict the patient survival and hence suggested as new prognostic markers [29].

*Melanoma*

In melanoma tissues, members of the 'MELmiR-17' panel were found to be predictors of the stage, recurrence, and survival. 'MELmiR-17' panel was found to be highly sensitive (93%) and specific (≥ 82%). Further, the panel was found to be superior to currently used serological markers for melanoma progression, recurrence and survival. This support that they would be ideally suited to monitor tumour progression in patients diagnosed with early metastatic disease (stages IIIa-c/IV M1a-b) and also able to detect relapse following surgical or adjuvant treatment [30].

*Lung Carcinoma*

Diagnostic assay based on hsa-miR-205 expression was studied in patients with cancer of lungs. The results found that miR-205 expression could able to discriminate the squamous from non-squamous non-small cell lung carcinoma [31].

*Hepatocellular Carcinoma*

Low level of miR-26 was an independent predictor of poor survival in patients suffering from

hepatocellular carcinoma. However, such patients responded well to interferon-α treatment, resulted in improved survival [32]. Therefore, miR-26 expression might be a useful biomarker to select patients who could benefit from an interferon-α therapy.

*Prostate Cancer*

The conventional tumor marker, prostate-specific antigen (PSA) has a low specificity and the optimal threshold for biopsy is unclear. It may also cause over diagnosis and treatment of indolent prostate cancers that may relapse in the post treatment and in majority of these patients the disease was more extensive than it appeared in the pre-treatment. Bryant et al. [33] demonstrated that changes in miR concentration may be detected in plasma and serum samples and may be useful as an aid in the diagnosis of prostate cancer. They further studied the use of miRNA for the diagnosis, staging and prediction of outcome and confirmed the association of serum-derived exosomes and microvesicles of miR-141 and miR-375 with metastatic prostate cancer. A total of 12 miRs were differentially quantified in prostate cancer patients compared with the controls, including 9 in patients without metastases. An analysis of the five selected miRs in urine samples found that miR-107 and miR-574-3p were quantified at significantly higher level in the urine of men with prostate cancer when compared with the controls. Similarly, study by Wang et al. [34] found the elevated miR-141 and miR-331-3p level in prostate cancer. The concentration of miR-141 was ~46-fold greater in men with metastatic prostate cancer. The sensitivity in predicting clinical outcome (progression vs. non-progressing) using the plasma miR-141 level was 78.9% [35]. miR-107 assay in plasma samples may be sensitive and specific enough for use as a single diagnostic test for non-metastatic prostate cancer. Furthermore, it may potentially improve the accuracy of conventional prostate cancer detection when combined with the PSA level.

*Bladder Cancer*

Urinary biomarkers are needed to improve the care and reduce the cost of managing bladder cancer. Cystoscopy and urine cytology are the currently used gold standards in the monitoring and diagnosis of bladder cancer. However, they struggle to identify both high and low-grade cancers due to the differing molecular pathways. Cystoscopy is an invasive and painful test that may not be able to detect small and/or flat tumors like carcinoma *in situ*. Detection of urinary miRs was able to detect the low- and high-grade cancers. miR panel such as miRs-15a/15b/24-1/27b/100/135b/203/212/328/1224 in patients with haematuria would detect 94% of urothelial cell carcinoma, while reducing cystoscopy rates by 26%. miR was stable within the urinary cells despite adverse handling and able to detect the differential expression of 10 miRs in cancer patients and controls. Among the panel tested, miR-1224-3p had the best result with specificity 83%, positive and negative predictive values of 83% and 75%, respectively and concordance of 77%. Further, the combination of miRs-135b/15b/1224-3p detected bladder cancer with a high sensitivity (94.1%) and sufficient specificity (51%) [36]. Similarly, Yamada et al. [37] reported that miR-96 and miR-183 in urine are promising tumor markers for urothelial carcinoma. miR-96, in particular may be a good diagnostic marker in combination with urinary cytology. The ratio of microRNA-126 to microRNA-152 in urine was studied to detect bladder cancer at a sensitivity of 82% and a specificity of 72%, with an AUC value of 0.768 [38].

*Pancreatic Cancer*

Serum miR-492 (75.5% sensitivity and 70.0% specificity) and miR-663a (85.7% sensitivity and 80.0% specificity) were significantly decreased in patients compared with controls and thus recommends as a strong potential to become a novel biomarkers for the early detection of pancreatic cancer [39]. Li et al. [40] reported that detection of elevated circulating serum level of miR-1290 has the potential for the early detection of pancreatic cancer. The elevated level could distinguish low-stage pancreatic cancer patients from the controls, better than CA19-9 levels. Furthermore, higher miR-1290, like CA19-9 levels, can predict the poorer outcome among the patients undergoing pancreaticoduodenectomy.

*Methods for Detecting the Extracellular Circulating miRNA*

Common methods for detecting miRNAs included the quantitative reverse transcriptase polymerase chain reaction (RT-qPCR), *in situ* hybridization, high throughput sequencing as well as the on microarray based profiling [41-44]. Among these methods, real-time RT-PCR is extremely sensitive and accurate; however it is a more expensive with low-throughput and the primer design can heavily influence the results. The short length and high sequence similarity among the miRNAs likely to contribute to the inconsistency of the measured results due to problems in designing specific primers for qPCR or probes for the microarrays. Technically challenging, low throughput and semi-quantitative were the limitations

found in the *in situ* hybridization method. Though the sequence-based methods allow the identification of unknown miRNAs expression at a low level, the technique is extremely expensive and time consuming. However, sequencing machines which are fast, accurate and the most promising had led to the discovery of new miRNAs in biological specimens.

*Conclusions and Future Prospects*

Detection of human cancers using biomarkers, especially at an early stage is urgently required to reduce the worldwide morbidity and mortality. The conventional circulatory tumor markers were failed to a great extends due to their low specificity to detect malignancy. Some of them are effective for monitoring patients with metastatic disease (eg. CA 125, PSA) or monitoring relapse from the chemotherapy (eg. CEA, PSA). Since miRNA has central role in the regulation of gene expression, they can be possibly developed as reliable diagnostic, predictive or prognostic biomarkers. The miRNA as biomarker succeeded some criteria needed for the tumor marker to be accepted for clinical practice such as stability in extracellular fluids and noninvasive nature (Figure 3). However, several fundamental issues associated with miRNA

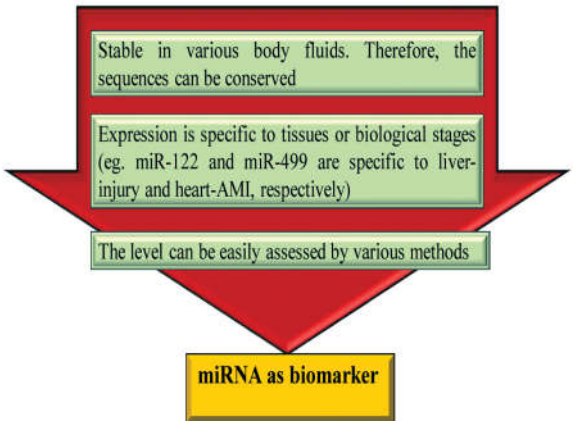


**Fig. 3:** Properties of microRNA (miRNA) to be selected as the biomarkers for cancer

measurements that still need to be addressed for their wide recommendation. Indeed, lack of consistency between reported studies certainly gives rise to some concern that may arise from pre-examination (sample selection or preparation), examination (experimental design) and post-examination (data analysis) phases. The methods that detect the extracellular miRNAs, currently uses no known extracellular housekeeping RNAs which can be used for the normalization of the procedure. Overall, this suggested the need for a more comprehensive validation and the development as well as optimization of an efficient, sensitive and reproducible detection method. Therefore, mi RNA expression to detect the cancer or to predict the response of different therapies needs to be further investigated and validated with respect to the conventional tumor markers.

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