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## Review Article

# MicroRNA Applications in Human Cancers

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## Abstract

**Over the last decade, a family of noncoding RNA molecules, including microRNA (miRNA) has been discovered. MiRNAs have been implicated as key regulators of different cellular processes many of which are associated with cancer. They contribute to the pathogenesis of human cancers; in addition, they may function as oncogenes or tumor suppressors in tumor development. The unique expression profile of microRNAs in different types and subsets of tumor, besides to their possible identification in biological fluids indicate that they might serve as molecular biomarkers for cancer diagnosis, prognosis, and prediction of therapeutic responses. However, they should achieve remarkable sensitivity and specificity, reproducibility and consistency between different studies. Accumulating evidence suggests that alterations of miRNAs expression may ultimately yield new therapeutic strategies against cancer or at least guide clinical decision making. The current review will give an overview on miRNAs, with a focus on their applications in human cancer diagnosis, prognosis, and therapy.**

**Keywords**: Cancer therapy; microRNA; tumorogenesis

## Introduction

After the completion of the human genome sequencing, it became clear that over ninety percent of this genome encode for RNA transcripts that are not translated to protein[(1)]. One such example is the microRNAs (miRNA) firstly discovered in 1993. In the last two decades, they have become the subject of several researches, which revealed a large number of information regarding miRNA biogenesis, function and significance in gene regulation. The biological significance of miRNAs was further tested in cancer biology and recent studies have demonstrated that dysregulation of miRNAs expression is characteristic of human malignancies, mainly through its direct regulation of different pathways involved in cancer development[(2-3)]. This review focuses on the current evidences for the involvement of miRNAs in the etiology of human cancer and the potential uses of miRNAs in human cancer diagnosis and therapy.

### Overview of miRNA history and biogenesis

In 2000, Zamore et al. studied the RNA interference (RNAi) process and described how double-stranded RNA fragments of

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21–23 nucleotides (nt) target mRNA cleavage and block gene expression[4]. Within the following years, a large pool of non-protein-coding RNAs (ncRNA) has been identified, and much attention has been focused on miRNAs because of their function as posttranscriptional modulators of gene expression during development and disease[5].

*MiRNA* genes encodes for long precursor molecules that are further processed to produce mature miRNAs. Two RNase III endonucleases, Drosha and Dicer, subsequently process the primary transcripts (or pri-miRNA) to generate mature miRNAs[6] (figure 1). In the nucleus, the primary transcripts contain one or more stem-loop structures of ~ 60-70 nt. The ribonuclease Drosha excises the stem-loop structure to form the precursor miRNA (or pre-miRNA)[7]. This pre-miRNA is actively exported to the cytoplasm by Ran-GTP and the export receptor Exportin-5[8]. In the cytoplasm, Dicer recognizes the double-stranded portion of the pre-miRNA, then, cleaves it generating a short RNA duplex (miRNA:miRNA*****)[6]. After untwisting, one RNA strand becomes the mature single-stranded miRNA, while the complementary strand, miRNA*****, is usually rapidly degraded.

The mature single-stranded miRNA forms a complex with proteins, termed the RNA-induced silencing complex (RISC); this silencing complex can bind to the target mRNA based on base pairing, causing inhibition of protein translation and/or degradation of the target mRNA (Figure 1)[9-10]. Such regulation leads to a decrease in protein level of the target gene while the number of mRNAs per cell is not affected. An alternate pathway emphasizes the role of miRNA in destabilization of mRNA containing adenine/uridine-rich element (ARE) in their 3` UTR region. By targeting this ARE, miRNA induces mRNA decay[11]. Furthermore, mRNA expression changes associated with miRNA may be mediated indirectly through their down-regulation of transcription factors, RNA regulating proteins, and proteins that will interact with other target protein. Subsequently, miRNAs may indirectly exert their regulatory effect by alteration of transcription of other gene, and levels of other mRNAs[12]. In humans, miRNAs mainly inhibit protein translation of their target genes, while degradation or cleavage of the mRNA is an infrequent process[6].

Currently, there are 2,555 mature human miRNA sequences listed in the miRNA registry (Sanger miRBase release 20; http://www.mirbase.org/). Computational predictions of miRNA targets suggest that each miRNA regulates tens or hundreds of protein coding genes. In fact, it is estimated that miRNAs may regulate up to two-thirds of the human genome[13]. This accentuates the important role of these miRNAs in downregulation of mRNA levels, and subsequently repression of translation of hundreds of genes, directly and indirectly[14].

**MiRNA and tumorogenesis**

The first link between miRNA and cancer was described by Calin et al. in 2002. They found that miR-15a and miR-16-1 were disrupted by translocations or deletions in chronic lymphocytic leukemia (CLL) patients. This genetic alteration was associated with decreased expression of the two mature miRNA. The authors suggested that these two miRNAs could be the tumor suppressors involved in the pathogenesis of CLL since they were downregulated or suppressed in most CLL cases[15]. MiRNAs are involved in vital cellular processes including the cell cycle control. Alteration in their expression might induce malignant transformation[16], impair response to DNA damage[17], and/or result into metastasis[18].

Several mechanisms have been suggested to explain the miRNA expression dysregulation that might lead to tumorogenesis including: 1) chromosomal abnormalities, as miRNAs are located in fragile regions of the genome. This was confirmed by a genetic study in ovarian carcinoma, breast cancer, and melanoma[19]; 2) mutations, as detected in an in vivo study on CLL[20]; 3) polymorphisms as described in lung cancer[21]; 4) defects in the miRNA biogenesis machinery, as supported by the changes in miRNA levels consequent to altered Drosha or Dicer activity in different tumor types[22-23]; and finally 5) epigenetic regulation, such as promoter methylation or histone acetylation. These mechanisms can modulate miRNA genes expression, since approximately half of these genes are rich in CpG islands, suggesting that they could be subjected to such regulatory mechanisms[24].

Several miRNAs has been correlated to specific DNA regions related to cancer development[25]. They have been classified as oncogenic, tumor-suppressive, or context-dependent miRNAs[26]. By acting as oncogenes or tumor suppressor genes, miRNAs can modulate the oncogenic potentiality or change the levels of specific tumor suppressor proteins. In cancers, oncogenic miRNAs are frequently overexpressed, while tumor suppressive miRNAs are deleted[26]. Mutation in the miRNA can disrupt its binding recognition site to its targets; it can also result in its activation as oncogene and/or repression if it is a tumor suppressor[27]. In addition, miRNAs might target epigenetic modifiers, resulting in widespread epigenetic alterations including methylation of promoters of other miRNAs that target oncogenes[26,28]. For instances, miR-21 is one of the microRNAs with a well established role as oncogene, their overexpression is associated with several human neoplasia. It has been considered a molecular prognostic marker for breast cancer. Increased miR-21 levels have been correlated to specific breast cancer pathological features, including advanced tumor stage and lymph node metastasis, in addition to poor patient's survival and prognosis[29].

**MiRNA applications in human cancer**

MiRNA profiling is now considered an interesting area of research to decipher their potential use in tumor classification, diagnosis and prognosis. With the advance of high-throughput sequencing, large scale profiling of miRNA became more easily assessed in normal and diseased tissues[30]. Genome-wide profiling showed that miRNA expression signatures (miRNome) allowed discrimination between different types of cancer with high accuracy. In addition, the tissue of origin of poorly differentiated tumors can be easily identified[31].

*i. Use of miRNA in cancer Diagnosis:*

Although pathology is considered the most useful diagnostic tool of cancer diagnosis, yet limitations of tissue biopsy necessitate the development of non-invasive biomarkers for some malignancies. The concept of circulating miRNA in cancer diagnosis is relatively new, however several studies demonstrated its feasibility for detection of malignant tumors[32]. Moreover, the use of miRNAs in diagnosis is more effective than mRNA because it is readily more stable. This enables their extraction in suitable amount from paraffin-embedded tissues, as well as from different biological fluids, and their quantification effectively with strong diagnostic and prognostic potentials[33]. Examples of circulating miRNAs involved in human cancer are summarized in table 1. One of the most important aspects of the miRNA fingerprints is not only to distinguish between normal and cancerous tissues but also to discriminate between different cancer subtypes.

**Table 1:** Circulating miRNA dysregulated in common human cancers

| Cancer type | Type of sample | miRNAs | Expression pattern | Prognostic value |
|---|---|---|---|---|
| Breast cancer | Serum<br><br>Whole blood | • miR-21,-106a,-155, -25, -223<br>• miR-126, -199a, -335<br>• - miR-195 | Upregulated<br>Downregulated<br>Upregulated | miR-155 associated to PR status.<br>miR-21, -10b associated to ER.<br>Reduced let-7a correlate with lymph node metastasis. |
| Hepatocellular carcinoma | Serum<br><br>Plasma | • miR-25, -375, let-7f, -21, -122, -223<br>• miR-21 | Upregulated | - |
| Prostate cancer | Serum | • miR-20b, -874, -1274, -1207, -93, -106a, -21<br>• miR-24, -223, -26b, -30c | Upregulated<br><br>Downregulated | miR-21 overexpression is associated to docetaxel resistance<br>Low miR-24 correlated to metastasis |
| Burkitt's & Hodgkin lymphomas | Serum | • miR-155 | Upregulated | - |
| ALL | Serum | • miR-125b-1 | Downregulated | - |
| CLL | Serum | • miR-15$^a$, -16-1<br>• miR-19b-1, -92-1 | Downregulated<br>Upregulated | - |
| Colorectal cancer | Plasma | • miR-221<br>• miR-143, -145 | Upregulated<br>Downregulated | Associated with poor overall survival |
| Pancreatic carcinoma | Serum<br>Plasma | • miR-141<br>• miR-21, -155, -196a | Upregulated | - |
| Papillary thyroid carcinoma | Serum | • miR-221, -222, -146 | Upregulated | - |
| NSCLC | Serum | • miR-486, -30d, -10b, -155<br>• miR-1, -499 | Upregulated<br>Downregulated | miR-486, -30d, -1, -499 expression correlate with overall survival<br>miR-10b correlated with lymph node Metastasis |
| HNSCC | Plasma<br>Serum | • miR-184<br>• miR-34, -10b, -155 | Upregulated<br>Upregulated | Not related to prognosis<br>Correlate with Metastasis |

ALL: acute lymphocytic leukemia; CLL: chronic lymphocytic leukemia; ER: estrogen receptor; HNSCC: Head and Neck Squamous Cell Carcinoma; NSCLC: Non-small cell lung cancer; PR: progesterone receptors [59-60].

For instance, miRNAs expression can differentiate between basal and luminal breast cancer subtypes, originating from epithelial and myoepithelial cells, respectively[34]. For example, miR-200 is upregulated in well-differentiated luminal breast. They directly target two transcription factors that control the epithelial-to-

mesenchymal (EMT) transition, an essential process that enables cancer cells to become motile, invasive, and more metatstatic[35]. On the other hand, miR-145 and miR-205, normally expressed in myoepithelial cells, are dramatically downregulated in basal breast cancer negative to estrogen, progesterone and HER2/Neu receptors, giving a clue for worst prognosis in this subtype[36]. MiR-205 expression can also discriminate between squamous and non-squamous nonsmall cell lung carcinoma[37]. MiRNA expression profile might be a useful tool in identifying the origin of tumors that are difficult to be determined when the tumor is associated with metastasis in multiple sites. Cancers of unknown primary origin account for approximately 4% of all malignancies and are associated with poor prognosis [38]. Therefore, specific miRNA pattern may be of benefit in tumor classification, diagnosis and subsequent treatment[31]. Moreover, early diagnosis of cancer is usually associated with better prognosis. MiRNAs have proved high effectiveness as biomarkers for an early diagnosis. For example, miR-205 and miR-21 overexpression in ductal adenocarcinoma precede phenotypic changes in the ducts[39].

The area of research in miRNA signature profiling and the identification of candidate miRNA in specific type of cancer have grown tremendously in the last few years. Despite the encouraging reports, miRNA profiling has several limitations including, identification of the ideal tool for assessment, application of adequate bioinformatics analysis (e.g. use of controls for normalization), achieving reproducibility and consistency between different studies as well as minimizing the cost of the test[40].

*ii. Use of miRNA in cancer prognosis and therapeutic response:*

miRNAs have also a role in prediction of cancer prognosis as well as diagnosis, and/or response to specific therapies. Several groups have reported success in utilizing miRNAs as prognostic markers to predict cancer outcome. Calin et al.[20] described a unique microRNA expression signature composed of 13 genes that were associated with prognostic factors and disease progression in CLL patients. Li et al.[41] described seven miRNAs signature that can predict overall survival and relapse-free survival in patients with gastric cancer. MiR-155 overexpression and let-7a downregulation were able to predict poor outcome in lung cancer[42]. Furthermore, low miR-191 and high miR-193a levels were associated with a significantly shorter survival time in melanomas[43]. MiRNAs profiling might also be important in prediction of response to therapies in malignancies. For example, hepatocellular carcinoma patients with low miR-26 responded well to treatment with interferon-α with an improved survival rate[44]. Moreover, high levels of miR-125b in breast cancer predict poor response to taxol-based treatments *in vitro*[45]. Another potential role for miRNAs is alteration of chemotherapeutic response. MiRNA repletion or silencing may be used to augment chemotherapeutic effect. For instances, targeting miR-21 has been implicated in chemotherapeutic response across several malignancies, i.e. breast cancer[46]. Silencing of miR-1 in lung cancer improve the sensitivity to traditional chemotherapeutics[47]. Additionally, miRNA signatures might guide chemotherapeutic decisions, as miRNAs profiling studies have been used for classification of patients with various type of cancer into responders and nonresponders to therapy[48]. The effect of miRNA is not only restricted to chemotherapy, but also it might extend to induction of sensitization for radiotherapy. MiRNAs may modulate the DNA damage response, thus sensitizing tumor cells to both chemotherapy and radiotherapy[13].

*iii. Use of miRNA in cancer therapy:*

MiRNA manipulation to be used in cancer therapy is considered an interesting area of research, although it is quite challenging. The usefulness of this approach is that it is not only targeting a single gene, but it is able to interact with multiple molecules, making them extremely efficient in regulating distinct biological processes related to cancer development and progression[31]. Two main principles of miRNA manipulation are involved in cancer therapy. These include either repletion of selective tumor-suppressive miRNAs or directed silencing or reduction of tumor promoting miRNAs[49]. Indirect therapeutic strategies involve the use of drugs to modulate miRNA expression by targeting their transcription and processing[31]. Taking into consideration the two main therapeutic uses of miRNAs, two forms of potent oligonucleotides have been generated: one targeting miRNAs, known as anti-miRs, and the second involves production of a synthetically active form of miRNAs, or miR mimics/mimetics[50].

Reintroduction of miRNA might help in inhibition of cancer. For instances, repletion of miR-15a and miR-16-1 in leukaemic cells (MEG01) induces apoptosis, and this causes inhibition of tumour growth in vivo in a xenograft model[51]. In this therapeutic approach, synthetic RNA duplexes are designed to mimic or re-express a miRNA restoring its endogenous functions. They are designed with modifications to ensure their stability and cellular uptake[50]. For example, subcutaneous administration of miR-34a that transiently inhibited human colon cancer progression in vivo[52]. Intranasal administration of an adenovirus expressing let-7a miRNA reduced tumor formation in a mouse lung cancer model[53]. However, it is important to underline the possible hazards in reintroducing a miRNA with a viral system. In addition, the site of integration is unpredictable, and there is always a risk of insertional mutagenesis and activation of proto-oncogenes[31]. Another drawback is that, although this method would replace the miRNA levels lost during disease progression, it will also result in the uptake by tissues that do not normally express the miRNA of interest, resulting in potential side effects[13].

On the other hand, the best-studied modality of miRNA therapy is mediated by targeting miRNA for suppression through the use of antimiRs. These molecules bind by complementary to their target miRNA, causing either their repression or complete silencing [13]. Recent data emphasized the potential use of anti-miR compounds that inhibit specific miRNAs, as a new class of drugs. The advantage of using this approach is that miRNAs are small and their sequence is known and highly conserved among species. This made them an attractive point from which drug development can start[50]. To achieve miRNA loss-of-function, chemically modified antimiR oligonucleotides (AMOs) have been developed[54]. For example, silencing oncogenic miR-21 with antisense oligonucleotides generates a proapoptotic and anti-proliferative response in vitro in different cellular models, and also reduces tumor development and metastatic potential *in vivo*[55]. In addition, miR-10b has shown a crucial role as metastmiR [MicroRNA playing a crucial role in the metastatic process] in breast cancer. Systemic treatment of tumor-bearing mice with miR-10b antagomirs [chemically engineered oligonucleotides able to silence endogenous miRNAs] suppresses breast cancer metastasis[56]. An example of a highly promising miRNAs in therapy is miRNA-122. MiR-122 is a hepatic-specific miRNA that has shown to be of critical importance for the replication of

hepatitis C virus (HCV)[57]. Subsequently, miR-122 was targeted using antisense oligonucleotides, this resulted in significant reduction of HCV viral load in a chimpanzee model of chronic HCV infection with minimal toxicity, suggesting that miR-122 might be used as antiviral therapy[58]. Subsequently, a human phase IIa trial was conducted using a modified miR-122 antagonist. This trial showed high efficacy of the anti-miR as antiviral agent, in addition to its high safety[13].

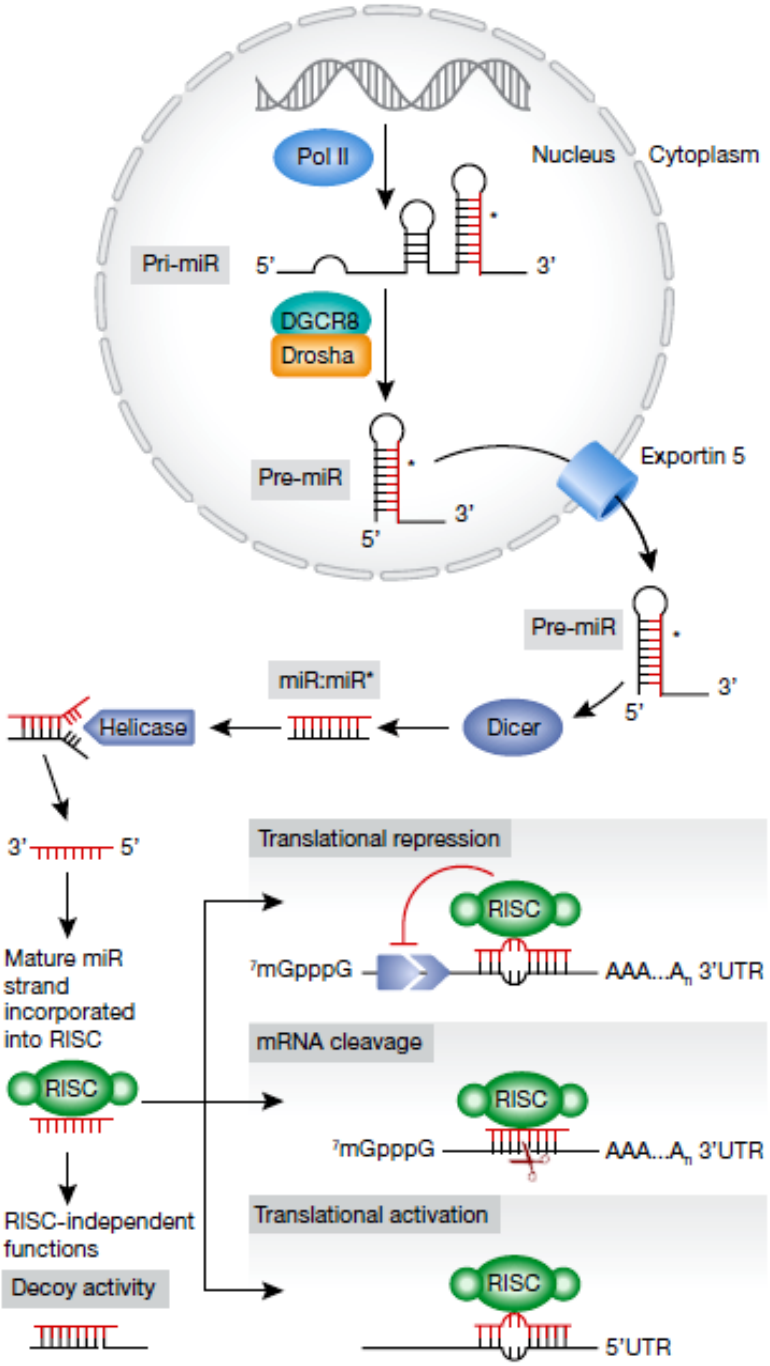


**Figure 1:** MiRNA biogenesis and mechanisms of action [Adapted from Iorio and Croce[31]]

## Conclusion

The role of miRNA in human cancer pathogenesis has provoked a significant number of researches. The high specificity of miRNAs makes these small molecules highly informative as cancer biomarkers. They are also considered potential markers for molecular analysis, since they can be extracted from a wide range of tissues as well as body fluids. The perspective of using miRNA in clinical practice is not restricted only to early detection of cancer but also as a putative therapeutic agent. However, before starting clinical trials on miRNAs, several problems should be addressed and solved. First, a full understanding of miRNAs role in cancer development should be accomplished. Secondly, extensive profiling of miRNA signature has to be achieved in normal and malignant tissues. Thirdly, data regarding the use of a specific miRNA as biomarker has to be reproducible in relation to a certain type of cancer. Fourthly, the possible application of miRNA molecules in cancer therapy needs further assessment to investigate how they might modulate cell growth and proliferation. In general, full understanding of the role of miRNAs will provide new insights on the molecular basis of cancers, and will help development of new biomarkers for cancer diagnosis, prognosis as well as new therapeutic agents.

## References


1. Mattick JS, Makunin IV. Non-coding RNA. Hum Mol Genet. 2006;15 Spec No 1:R17-29.
2. Almeida MI, Reis RM, Calin GA. MicroRNA history: discovery, recent applications, and next frontiers. Mutat Res. 2011;717(1-2):1-8.
3. O'Donnell KA, Mendell JT. Dysregulation of microRNAs in human malignancy. In: Appasani K, Altman S, Ambros VR, editors. MicroRNAs: Cambridge University Press; 2007.
4. Zamore PD, Tuschl T, Sharp PA, Bartel DP. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell. 2000;101 (1):25-33.
5. Martens-Uzunova ES, Olvedy M, Jenster G. Beyond microRNA--novel RNAs derived from small non-coding RNA and their implication in cancer. Cancer Lett. 2013; 340 (2):201- 211.
6. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116(2):281- 297.
7. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Rådmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003; 425 (6956):415- 419.
8. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science. 2004; 303 (5654):95-98.
9. Sassen S, Miska EA, Caldas C. MicroRNA: implications for cancer. Virchows Arch. 2008; 452 (1):1-10.
10. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev. 2003; 17 (24):3011-6.
11. von Roretz C, Gallouzi IE. Decoding ARE-mediated decay: is microRNA part of the equation? J Cell Biol. 2008;181 (2):189-194.
12. Liu X, Chen Z, Yu J, Xia J, Zhou X. MicroRNA profiling and head and neck cancer. Comp Funct Genomics. 2009: 837514.
13. Nana-Sinkam SP, Croce CM. Clinical applications for microRNAs in cancer. Clin Pharmacol Ther. 2013; 93 (1):98-104.
14. Selbach M, Schwanhäusser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. Nature. 2008; 455 (7209):58-63.
15. Calin GA1, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. Frequent

deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2002;99 (24):15524-15529.
16. Bueno MJ, Malumbres M. MicroRNAs and the cell cycle. Biochim Biophys Acta. 2011;1812(5):592-601.
17. Wan G, Mathur R, Hu X, Zhang X, Lu X. miRNA response to DNA damage. Trends Biochem Sci. 2011;36(9):478- 484.
18. White NM, Fatoohi E, Metias M, Jung K, Stephan C, Yousef GM. Metastamirs: a stepping stone towards improved cancer management. Nat Rev Clin Oncol. 2011;8(2):75-84.
19. Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, Liang S, Naylor TL, Barchetti A, Ward MR, Yao G, Medina A, O'brien-Jenkins A, Katsaros D, Hatzigeorgiou A, Gimotty PA, Weber BL, Coukos G. microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci U S A. 2006;103 (24):9136- 9141.
20. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med. 2005;353 (17):1793- 1801.
21. Hu Z, Chen J, Tian T, Zhou X, Gu H, Xu L, Zeng Y, Miao R, Jin G, Ma H, Chen Y, Shen H. Genetic variants of miRNA sequences and non-small cell lung cancer survival. J Clin Invest. 2008;118 (7):2600- 2608.
22. Nakamura T, Canaani E, Croce CM. Oncogenic All1 fusion proteins target Drosha-mediated microRNA processing. Proc Natl Acad Sci USA. 2007; 104(26):10980-5.
23. Merritt WM, Lin YG, Han LY, Kamat AA, Spannuth WA, Schmandt R, Urbauer D, Pennacchio LA, Cheng JF, Nick AM, Deavers MT, Mourad-Zeidan A, Wang H, Mueller P, Lenburg ME, Gray JW, Mok S, Birrer MJ, Lopez-Berestein G, Coleman RL, Bar-Eli M, Sood AK. Dicer, Drosha, and outcomes in patients with ovarian cancer. N Engl J Med. 2008;359(25):2641-50.
24. Weber B, Stresemann C, Brueckner B, Lyko F. Methylation of human microRNA genes in normal and neoplastic cells. Cell Cycle. 2007;6(9):1001- 1005.
25. Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. Nat Rev Cancer. 2010;10(6):389-402.
26. Kasinski AL, Slack FJ. Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. Nat Rev Cancer. 2011;11(12):849- 864.
27. You JS, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell. 2012;22(1):9-20.
28. Fabbri M, Calin GA. Epigenetics and miRNAs in human cancer. Adv Genet. 2010;70:87-99.
29. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, Zeng YX, Shao JY. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA. 2008;14 (11):2348- 2360.
30. Farazi TA, Horlings HM, Ten Hoeve JJ, Mihailovic A, Halfwerk H, Morozov P, Brown M, Hafner M, Reyal F, van Kouwenhove M, Kreike B, Sie D, Hovestadt V, Wessels LF, van de Vijver MJ, Tuschl T. MicroRNA sequence and expression analysis in breast tumors by deep sequencing. Cancer Res. 2011;71(13): 4443- 4453.
31. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med. 2012;4(3):143- 159.
32. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer

detection. Proc Natl Acad Sci U S A. 2008;105(30):10513- 8.
33. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer. 2011;11(6):426- 437.
34. Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin SF, Dunning MJ, Barbosa-Morais NL, Teschendorff AE, Green AR, Ellis IO, Tavaré S, Caldas C, Miska EA. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. Genome Biol. 2007;8 (10):R214.
35. Gregory PA, Bracken CP, Bert AG, Goodall GJ. MicroRNAs as regulators of epithelial-mesenchymal transition. Cell Cycle. 2008;7(20):3112-8.
36. Sempere LF, Christensen M, Silahtaroglu A, Bak M, Heath CV, Schwartz G, Wells W, Kauppinen S, Cole CN. Altered MicroRNA expression confined to specific epithelial cell subpopulations in breast cancer. Cancer Res. 2007;67(24):11612- 11620.
37. Lebanony D, Benjamin H, Gilad S, Ezagouri M, Dov A, Ashkenazi K, Gefen N, Izraeli S, Rechavi G, Pass H, Nonaka D, Li J, Spector Y, Rosenfeld N, Chajut A, Cohen D, Aharonov R, Mansukhani M. Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. J Clin Oncol. 2009; 27 (12): 2030- 2037.
38. Oien KA, Evans TR. Raising the profile of cancer of unknown primary. J Clin Oncol. 2008;26 (27): 4373- 4375.
39. du Rieu MC, Torrisani J, Selves J, Al Saati T, Souque A, Dufresne M, Tsongalis GJ, Suriawinata AA, Carrère N, Buscail L, Cordelier P. MicroRNA-21 is induced early in pancreatic ductal adenocarcinoma precursor lesions. Clin Chem. 2010; 56(4): 603- 612.
40. Pritchard CC, Cheng HH, Tewari M. MicroRNA profiling: approaches and considerations. Nat Rev Genet. 2012; 13 (5):358-69.
41. Li X, Zhang Y, Ding J, Wu K, Fan D. Survival prediction of gastric cancer by a seven-microRNA signature. Gut. 2010;59 (5): 579-85.
42. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell. 2006;9(3):189- 198.
43. Caramuta S, Egyhazi S, Rodolfo M, Witten D, Hansson J, Larsson C, Lui WO. MicroRNA expression profiles associated with mutational status and survival in malignant melanoma. J Invest Dermatol. 2010;130(8):2062- 2070.
44. Ji J, Shi J, Budhu A, Yu Z, Forgues M, Roessler S, Ambs S, Chen Y, Meltzer PS, Croce CM, Qin LX, Man K, Lo CM, Lee J, Ng IO, Fan J, Tang ZY, Sun HC, Wang XW. MicroRNA expression, survival, and response to interferon in liver cancer. N Engl J Med. 2009;361(15):1437- 1447.
45. Zhou M, Liu Z, Zhao Y, Ding Y, Liu H, Xi Y, Xiong W, Li G, Lu J, Fodstad O, Riker AI, Tan M. MicroRNA-125b confers the resistance of breast cancer cells to paclitaxel through suppression of pro-apoptotic Bcl-2 antagonist killer 1 (Bak1) expression. J Biol Chem. 2010; 285 (28): 21496- 21507.
46. Gong C, Yao Y, Wang Y, Liu B, Wu W, Chen J, Su F, Yao H, Song E. Up-regulation of miR-21 mediates resistance to trastuzumab therapy for breast cancer. J Biol Chem. 2011; 286 (21):19127-19137.
47. Nasser MW, Datta J, Nuovo G, Kutay H, Motiwala T, Majumder S, Wang B, Suster S, Jacob ST, Ghoshal K. Down-regulation of micro-RNA-1 (miR-1) in lung cancer. Suppression of tumorigenic property of lung cancer cells and their sensitization to doxorubicin-induced apoptosis by miR-1. J Biol Chem. 2008;283(48):33394-333405.
48. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, Yuen ST, Chan TL, Kwong DL, Au GK, Liu CG, Calin GA, Croce CM, Harris CC. MicroRNA expression profiles associated with prognosis and therapeutic outcome in

colon adenocarcinoma. JAMA. 2008;299 (4):425- 436.
49. Aagaard L, Rossi JJ. RNAi therapeutics: principles, prospects and challenges. Adv Drug Deliv Rev. 2007;59(2-3):75-86.
50. van Rooij E, Purcell AL, Levin AA. Developing microRNA therapeutics. Circ Res. 2012;110(3):496-507.
51. Calin GA, Cimmino A, Fabbri M, Ferracin M, Wojcik SE, Shimizu M, Taccioli C, Zanesi N, Garzon R, Aqeilan RI, Alder H, Volinia S, Rassenti L, Liu X, Liu CG, Kipps TJ, Negrini M, Croce CM. MiR-15a and miR-16-1 cluster functions in human leukemia. Proc Natl Acad Sci U S A. 2008;105(13):5166- 5171.
52. Tazawa H, Tsuchiya N, Izumiya M, Nakagama H. Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. Proc Natl Acad Sci U S A. 2007;104(39):15472-15477.
53. Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L, Weidhaas JB, Brown D, Bader AG, Slack FJ. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. Cell Cycle. 2008;7(6):759- 764.
54. Weiler J, Hunziker J, Hall J. Anti-miRNA oligonucleotides (AMOs): ammunition to target miRNAs implicated in human disease? Gene Ther. 2006;13 (6):496-502.
55. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. Oncogene. 2007;26(19):2799- 2803.
56. Ma L, Reinhardt F, Pan E, Soutschek J, Bhat B, Marcusson EG, Teruya-Feldstein J, Bell GW, Weinberg RA. Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. Nat Biotechnol. 2010;28(4):341- 347.
57. Jopling CL, Schutz S, Sarnow P. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. Cell Host Microbe. 2008;4(1):77-85.
58. Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, Kauppinen S, Ørum H. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science. 2010;327(5962):198-201.
59. Di Leva G, Croce CM. miRNA profiling of cancer. Curr Opin Genet Dev. 2013; 23(1): 3-11.
60. Ahmad J, Hasnain SE, Siddiqui MA, Ahamed M, Musarrat J, Al-Khedhairy AA. MicroRNA in carcinogenesis & cancer diagnostics: a new paradigm. Indian J Med Res. 2013;137(4):680- 694.