REVIEW ARTICLE

The role of exosomes derived miRNAs in cancer

Shahid Hussain,[1] Syeda Eeman Zahra Bokhari,[2] Xing Xing Fan,[3] Shaukat Iqbal Malik[4]

Abstract

Exosomes are 20-150nm cell secreting nano-bodies that helps in the transportation of various biomolecules, including micro ribonucleic acid (miRNA) in the human body during both normal and diseased conditions. The current review was planned to summarise the role of miRNA carried by circulatory exosomes in cancer. miRNA is responsible for contribution in cancer, regulation of gene expression, interfering in biological pathways, gene silencing or amplification, and also has a role in cancer resistance. (miRNA) plays a dynamic role in this process by regulating the genes related to drug resistance, cell proliferation, cell cycle and apoptosis through a tissue-specific fashion. Owing to its significances, micro ribonucleic acid has been reported to be the key regulator of cancer, metastasis and also a factor in cancer resistance, and is a better source of possible potential diagnostic biomarkers. Though many studies have explored the biological roles of RNAs in cancer, many facts are needed to be investigated for clinical applications.

Keywords: Exosomes, miRNAs, Cancer.

DOI: https://doi.org/10.47391/JPMA.398

Introduction

Exosomes are a type of extracellular vesicles enclosed within an outer membrane usually 30-15nm in diameter. They are also called intraluminal vesicles and are produced by most of the cell types, and, therefore, can be isolated from about all of the body fluids, including plasma, urine, semen, saliva, bronchial fluid, cerebral spinal fluid (CSF), breast milk, serum, amniotic fluid, synovial fluid, tears, lymph, bile and gastric acid.[1] One of the major cellular functions is the synthesis and transportation of biomolecules, such as the transportation of insulin in the bloodstream. Micro ribonucleic acid (miRNAs) are often called the regulators of the genome.[2] Mast cells were the first reported cells to carry nucleotide sequences in exosomes, which further led to the discoveries reporting the miRNAs and messenger RNAs (mRNAs) as contents of exosomes. Though the exact mechanism has not been reported, the exosomes are said to carry specific miRNAs and mRNAs only, which enhances the probability of its clinical application as diagnostic biomarkers. Moreover, packaging into the exosome provides the miRNA the safe capping for its transfer to various parts of the body. Thus, the secretion of miRNAs through exosomes in extracellular fluids, and their functions provide an insight into their role as potential biomarkers.[3] The information transmission and biomolecular transportation between the mammalian cells was discovered far before the discovery of the exosomes, but the underlying mechanisms remained a mystery. It was reported in 1983 that as reticulocytes in the blood matured into erythrocytes, transferrin receptors were released alongside through the functioning of small vesicles about 50nm in diameter.[4] In 1987, two scientists proposed the term "exosomes" for these vesicles. In 2012, a Nobel Prize was awarded to three scientists "for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells". These discoveries led to future studies about the exosomal mechanism of action in cellular trafficking and highlighted their role as biomarkers for diagnostic and therapeutic applications.[3]

miRNAs as Contents of Exosome

The miRNAs are being widely studied as biomarkers and signalling vehicles.[5] It was first discovered in 2007 that the exosomes also transferred functional RNAs, while further studies identified several protein and mRNAs that are carried by exosomes. It was later proposed that though the exosomes have a limited carrying capacity of functional RNAs, the majority percentage among these functional RNAs are miRNAs.[6]

Evolutionary Conservation

The miRNAs are also attributed to evolutionary conservation, as their functional targets are explored. The miRNA knockout animals frequently lack phenotypes, but their conservation in many subjects indicates that they confer certain selective advantages in the subjects. Another significant consideration about miRNAs is that they act through specific feedback loops within the gene regulatory networks, AND do not function individually.[7]

[1,2,4]Department of Biosciences, Capital University of Science and Technology, Islamabad, Pakistan, [3]State Key Laboratory of Quality Research in Chinese Medicines, Macau University of Science and Technology, Macau.

Correspondence: Shaukat Iqbal Malik. Email: iiuibiotech@gmail.com

Canalisation, a process of "stabilisation of biological outcomes despite genetic, environmental and stochastic perturbations" is the function performed by miRNAs, which is why it causes evolutionary conservation. The canalisation is achieved by two processes, i.e., redundant components and regulatory loops. Therefore, miRNAs ensure that minor genetic alterations caused by the environment are not conserved in the genetic makeup, thus protecting the organisms from harmful mutations and alterations.[8]

Role of miRNAs in Normal Physiology and Homeostasis

Approximately 60% of human protein-coding genes are potential targets for miRNAs, and the human body has 1,917 precursor miRNAs and 2,654 mature miRNAs.[9] The miRNAs regulate post-transcription modifications, regulating the protein synthesis. Therefore a variety of the biological processes are under the influence of miRNA regulations.[6] Animal development, cell differentiation and homeostasis are some of the vital cellular processes regulated by miRNAs which are associated with two major proteins; Drosha and Dicer, which are vital in embryonic development and other nuclear processes, such as the deletion of phenotypes in subsequent generations.[10]

Mechanism of miRNA

The functioning ability of miRNA is achieved by its binding with a protein called the Argonaute (AGO) protein. The structure of AGO protein comprises four characteristic domains within a single polypeptide chain: the amino-terminal (N) domain, the Piwi-Argonaute-Zwille (PAZ) domain, the middle (MID) domain, and the P-element-induced wimpy testes (PIWI) domain. The N-PAZ and MID-PIWI domains are joined by linker domains L1 and L2. Each of these domain units makes each of the two lobes of the AGO protein where the 5' ends of the miRNA is recognised and held by the MID-PIWI domain lobe, while the 3' end of miRNA is recognised and held by the N-PAZ domain lobe.[11] Four AGO proteins are encoded by the mammalian genome, but, among them, AGO2 protein is the most significant and performs the major role in cleaving the target molecule complementary to the miRNA strand and the regulation of miRNAs.[12] The loading of miRNA into specific AGO proteins can be due to functional preference or sometimes they may be loaded into any AGO proteins when convenient. The target site identification for the miRNA is achieved by the seed region's complementarity to the 3' untranslated region of mRNA.[13] The two prime features of a strong seed matching region on mRNA are the bases of mRNA that are complementary to the 2-8 nucleotide of miRNA and those that have an adenine to bind with the 1st nucleotide of miRNA called t1A' and this 't1A' is recognised by the binding domain of AGO protein.[14] Target site identification is a two-step process to achieve a specific conformation ideal for the reaction. This conformation is there so that the MID and PIWI domains of the AGO protein may organise the first 2-6 nucleotides of the seed region of miRNA into a stacked, helical conformation, and the 2-4 nucleotides get exposed to the solvent. This initial confirmation creates a weak metastable bond between the target region of mRNA and the seed region of miRNA and this bond is strengthened according to the presence of the two features of a strong seed matching region that has been mentioned above. Otherwise, the AGO protein disassociates the mRNA from the miRNA completely or partially. Thus the AGO-miRNA complex binds to the 3' untranslated region of the mRNA it leads to gene silencing and transcriptional inactivation, eventually leading to the decay of the mRNA as it is not expressed. Though the target site specification mechanism exists for

Table-1: Micro ribonucleic acids (miRNAs) participating in metabolic homeostasis along with their targets and mechanism of action.[27]

miRNA	Target Tissue	Target Gene	Action Pathway
miR-33ab	Liver	ABCA1	Increase in HDL, Antagonism of VLDLs
miR-103/107	Liver, white adipose	CAV1	Promotion of Insulin Resistance along with Serum Glucose Level
miR-143	Liver	ORP8	Impairment of Glucose Homeostasis, Transgenic expression
miR-802	Liver	HNF1b/ TCF2	Dysregulation of Hepatic Insulin Sensitivity and Glucose Tolerance
miR-378	Brown adipose, skeletal muscle, white adipose, liver	CRAT, MED13, Pde1b	Antibiotic effect through expression specific to adipose cells, promoted energy expenditure
miR-133	Brown adipose	PRDM16	Impairment of Differentiation of Brown Adipocyte and Thermogenic capability
let-7	Skeletal muscle, liver, pancreas	IGF1R, INSR, IRS2, HMGA2	Impairment of Insulin Sensitivity along with Insulin Secretion
miR-375	Pancreas	Various	Promotion of Beta Cells Mass
miR-7	Pancreas	SCNA	Down regulation of Insulin Secretion
miR-200	Pancreas	DNAJC3, JAZF1, RPS6KB1, XIAP, ZEB1	Promotion of Beta Cell Apoptosis

HDL: High-density lipoprotein; VLDL: Very low-density lipoprotein.

the miRNA action, a single miRNA can regulate hundreds of genes, and multiple miRNAs can regulate a similar gene.[15] Moreover, miRNA individually or in clusters can regulate entire cellular pathways. In addition, cooperative repression of gene silencing can be achieved when miRNAs start binding to the neighbouring target sites one single target mRNA, explaining the function of the weaker seed region binding sites.[16] Some of the miRNAs participating in metabolic homeostasis along with their targets and their mechanism of action are shown in Table-1.

Regulation Mechanism of miRNAs in Cancer

As the functioning mechanisms of miRNA were identified, so was its role in malignancies. Chromosomal abnormalities, transcriptional control changes, epigenetic changes and defects in the miRNA biogenesis machinery are the processes through which miRNA controls malignancies in the state of cancer.[9] The abnormal expression of miRNA in malignant cells is owed to either the abnormality in the copy number of miRNAs produced by the genome, or the variation in the location of production leading to the amplification, deletion or translocation of miRNAs. The gene location alteration in malignant cells was first observed when the cells of B-cell chronic lymphocytic leukaemia were analysed and showed a loss of miR-15a/16-1 cluster gene located at chromosome 13q14. In addition, a gene location change was also observed in lung cancer patients, where decreased expression of miRNA was attributed to the deletion of miR-143 and miR-145 genes located at chromosome 5q33. At the same time, the cells of B-cell lymphomas and lung cancers showed gene amplification of miR-17-92 cluster gene, resulting in the over-expression of miRNA in tumour cells.[9]

Regulation of Transcription by miRNAs

Various transcription factors control the expression of miRNA, therefore it can be inferred that abnormal regulation by transcription, such as c-Myc and p53, can also cause abnormal expression of miRNA in malignant cells. It was discovered that cell proliferation and apoptosis were highly regulated frequently due to transcription factor c-Myc, which, in turn, up-regulates the oncogenic miR-17-92 cluster. In addition to the up-regulation of oncogenic clusters, c-Myc also down-regulates the expression of tumour-suppressive miRNAs, such as mir-15a, miR-26, miR-29, mir-30 and let-7 families.[9]

Abnormal Regulation of Epigenetic Marks

Another established feature of malignant cells is the abnormal modification of epigenetic marks, including deoxyribonucleic acid (DNA) hypomethylation, abnormal DNA hypermethylation of tumour suppressor genes and disturbance in histone modification. Similar to the protein-coding genes, the miRNA coding genes are also exposed to the epigenetic mark and changes. Proving this hypothesis, it was found that CpG methylation causes the silencing of miRNA-223 through a protein fusion called acute myeloid leukaemia-1/ myeloid translocational gene 8 (AML1/MTG8). The MTG8 protien is also called ETO. In another investigation, T24 bladder cells showed a significant up-regulation of 17 out of 313 human miRNAs when they were exposed to DNA methylation and histone acetylation inhibitors. Among the up-regulated miRNAs in these cells was miRNA-127, which is usually a non-expressive miRNA embedded in a CpG island, but it showed significantly high expression in cancer cells, resulting in loss of expression in proto-oncogene B-cell lymphoma 6 (BCL6). Therefore, it can be inferred that activation of epigenetic changes and marks can up-regulate the miRNAs and their expression to suppress tumours and vice versa.[9]

Major Regulators for Cell Proliferation

The key regulatory family of cell proliferation is the E2 factor (E2F) protein family, which regulates it through a cell-cycle-dependent manner and this protein family is further regulated by miRNAs. The member of this protein family E2F1 is a tumour suppressor which causes the transcription of target genes between the cell cycle phase G1 and phase S. The c-Myc transcription factor promotes the expression of E2F1 through a positive feedback loop, while this loop is blocked by miRNA-17-92 when c-Myc activates it. Thus, E2F1 is not expressed despite having been activated by c-Myc. Other than E2F1, E2F2 and E2F3 translations are also regulated by miRNA-17-92 cluster and, in turn, the E2F transcription factor family can prompt miR-17-92 cluster expression. Therefore, it can be inferred that normal cell cycle progression is significantly regulated by the feedback mechanism between miR-17-92 cluster and the E2F family under normal conditions and cell proliferation can be induced by the disruption in this feedback mechanism between them in a cancerous state.[9] Another method of regulation of cell proliferation by miRNAs is by their regulation of various signalling pathways. For instance, miR-486, altogether down-regulated in cells of lung cancer, was found to influence cell multiplication and relocation through insulin-like growth factor (IGF) and PI3K flagging pathways by focussing on IGF1, IGF1R and p85α.[17]

Cell Death Resistance

Inhibition of apoptosis is another factor responsible for cancer progression which is regulated by miRNAs.[18]

Multiple mechanisms are adopted by malignant cells to achieve this. The most common of these is the loss of tumour suppression activity of p53. Other mechanisms adopted by the cancerous cells to evade apoptosis include the up-regulation of anti-apoptotic regulators, suppression of pro-apoptotic factors and inhibition of death pathway induced by extrinsic ligands, which are all regulated by miRNAs.[9] Various miRNAs are being regulated by p-53 functions, while the miRNAs also control p53 levels through a feedback mechanism. It was identified by a study that p53 degradation is inhibited in multiple myeloma by the activation of miRNA-192, miRNA-194, and miRNA-215, which, in turn, bind to the miRNA or mouse double minute 2 (MDm2), causing its suppression.[19] Thus, the three miRNAs are considered to play a significant role in multiple myeloma, as they act as positive regulators of p53. The anti-apoptotic regulators, such as B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xL), and pro-apoptotic factors, such as Bax, Bim and Puma play a significant role in cell death, which are significantly regulated by miRNAs in disease states. An example of this is miRNA-15a and miRNA-16-1 which cause an increase in Bcl-2 expression when these miRNAs are down-regulated in the state of chronic lymphocytic leukaemia.[9]

Invasion and Metastasis

Metastasis is a dynamic and complex biological mechanism comprising some sequential steps. An early phase of metastasis is characterised by the loss of cell adhesion called the epithelial-mesenchymal transition (EMT), caused by the down-regulation of E-cadherin and activation of genes associated with motility and invasion. Some key transcription factors, like Zinc finger E-box binding (ZEB), Zinc finger protein SNAI1 (sometimes referred to as Snail) and Twist-related protein 1 (TWIST1) regulates EMT through signalling pathways, such as transforming growth factor (TGF)-β. Various researches have reported evidence on the control of miRNAs over the process of cancer metastasis and in particular its early significance phase EMT. It was reported that EMT was induced to cause cancer metastasis as the TGF-β-regulated miRNAs were studied in TGF-β signalling, causing an advancement in malignancies in disease states. One such miRNA involved is the miRNA-155, which is transcriptionally activated by TGF-β/SMAD4 (SMAD family member 4, Mothers against decapentaplegic homolog 4) signalling causing metastasis and cancer progression.[9]

Angiogenesis

The process of development of new blood vessels from the pre-existing vessel is known as angiogenesis and this process is considerably increased to satisfy the increased requirements of nutrition and oxygen to the tumour cells as the cell division is abnormally increased. Despite increased angiogenesis, due to excessive cell division, the tumour region is mostly surviving in an oxygen-deprived state called hypoxia, which is a significant factor in the maintenance of the environment permissible for the growth of a tumour. The body produces transcription factors in response to hypoxia, called a hypoxia-inducible factor (HIF), which regulates a variety of other genes, including those of miRNAs. A significant angiogenic factor required for the promotion of vascular growth is the vascular endothelial growth factor (VEGF). It has been reported that various miRNAs target HIF and VEGF in the state of cancer to significantly increase angiogenesis.[9] One such miRNA which is released in the state of hypoxia, and regulated by HIF and VEGF, is miRNA-220 whose increased expression has been observed to increase the synthesis of capillary-like structures from the venous endothelial cells through a VEGF-dependent cell migration in normoxic human umbilical vein. A decrease or blockage in the expression of miRNA-220 reverses the process. The various miRNAs involved in tumour suppression or progression in colorectal cancer through regulation of various transcription factors are known (Table-2).[20]

Circulating miRNAs as Cancer Diagnostic Biomarkers

With each passing day, newer interventions are made regarding cancer diagnosis and treatment, including the

Table-2: Micro ribonucleic acids (miRNAs) involved in colorectal cancer which can act as diagnostic cancer biomarkers.[20]

miRNA	Target Gene	Regulation Abnormality	Dysregulation Effect
miR-155	CTHRC1	Downregulation	Decreased Cell Invasions, Increased cell cycle continuity and apoptosis
miR-205-5p	ZEB1	Downregulation	Impairment of epithelial to mesenchymal conversion
miR-18a	CDC42	Downregulation	Impairment of tumour cell growth
miR-26a	PDHX	Upregulation	Impairment of Glucose Metabolism
miR-221	TP53INP1	Upregulation	Increased Cell Invasions, Impairment of Apoptosis
miR-106a	PTEN	Upregulation	Increased Cell Invasions, Impairment of Apoptosis
miR-17-3p	Par4	Upregulation	Increased Cell Invasions, Impairment of Apoptosis

diagnosis through miRNAs. The basic principle of this miRNA-based diagnosis is that, as mentioned earlier, the expression level of miRNAs is considerably altered in abnormal cells leading to various physiological conditions, such as diseases, i.e., aging, diseases and cancers,[21] and this alteration can be detected, which can produce a signal for abnormality. Classifying these disease-causing miRNAs which were usually over-expressed in disease states, those which caused cancer, and play a role in tumour initiation and progression were termed oncogenes or "oncomiRs", whereas those having beneficial effects in cancer or that decreased the process of metastasis, were termed tumour-suppressive miRNAs.[22] These miRNAs are usually packaged in exosomes or other extracellular vesicles and are present in most body fluids in detectable quantity and form and therefore, they can act as significant cancer biomarkers.[23] Thus, liquid biopsies can be performed on various body fluids, such as peripheral blood, saliva, CSF, ascites, urine, breast milk and semen, allow for miRNA detection to detect cancer miRNA biomarkers.[24]

Contribution of Circulating miRNAs to Diagnose Tumour Metastasis

The progression of a tumour to various sites in the body is the major cause of poor survival rate and poor treatment outcomes in cancer patients, and there are no viable biomarkers to diagnose this as well. An example of this type of miRNA that can be used as a cancer biomarker is miRNA-497-5p, whose expression is considerably decreased in primary tumour tissues, metastatic tissues and serum in osteosarcoma patients compared to healthy patients. It produces cancerous effects by down-regulating target genes, like insulin-like growth factor-1 receptor (IGF-1R), Vascular endothelial growth factor (VEGF), angiomotin (AMOT), and CDK-interacting protein 1 (p21) which inhibit osteosarcoma cell proliferation, migration and invasion, and enhance apoptosis. Other effects of osteosarcoma attributed to miRNA-497-5p are clinical stage, distant metastasis and promoted cisplatin resistance. Another example is miRNA205-5p in bladder cancer, which was related to tumour stage and pathological grade in patients.[25]

Therapeutic Potential of miRNAs

The two mechanisms through which miRNAs can play a role in causing disease is either through an abnormal disease-causing expression or a complete loss of expression to perform normal activities. In both cases, two strategies can be adopted to achieve therapeutic targets. The first is the application of nucleotide strands of virus vectors to block the abnormal expression or compensate for missing expression. The second involves targeting the transcription and functioning of miRNAs through drugs to produce a normal expression of miRNA. Antisense oligonucleotides, small RNA inhibitors, miRNA sponges and miRNA masks can be employed to block the defective miRNA expression, whereas synthetic miRNA (miRNA mimic) or viral vectors consisting of effective miRNA genes, can be used to restore down-regulated miRNAs or missing miRNA expression. Small miRNA inhibitors perform their function on the transcriptional level and inhibit the conversion of defective pre-miRNA into mature miRNA. Antisense oligonucleotides work on the mature miRNA by destroying the defective miRNA or converting it back into the bound duplex to inhibit its expression. The application of miRNA masks is at the functional level, where they effectively bind to the 3' UTR (untranslated region) of the target mRNA, inhibiting the defective miRNA to the target region and this binding can be achieved with greater efficiency through miRNA sponges.[26]

Conclusion

The miRNA-based biomarkers are among some of the most noteworthy therapeutic and diagnostic strategies being explored in the field of oncology and other diseases. Moreover, exosome packaging of miRNAs makes them even more efficient in their function, as they are transported to various target sites in the body in a protective encapsulation from other cellular enzymes. The most critical role of miRNAs is in the process of 'gene silencing' where it binds to the 3' UTR region of mRNA of the target gene and impairs the protein synthesis procedure, thus affecting gene expression. Through this mechanism, it further causes various effects in terms of gene amplification or deletion, transcriptional regulation, epigenetic regulation and the regulation of biological pathways. The effects attributed to miRNAs in cancer

Table-3: Micro ribonucleic acids (miRNAs) involved in drug resistance in various cancer tumours.[28]

miRNA	Target Gene/Protein	Target Drug	Cancer Type
miRNALet-7b	ER-α36	?/TAM	Breast
	IMP-1	+/Taxanes	Ovarian
miRNA-Let-7c	ABCC2, BCL-XL	?/CDDP	NSCLC
miRNA-9	BRCA1	?/CDDP	Ovarian
miRNA- 17-5p	Beclin1	?/PTX	Lung
	PHIPP2	+/Topotecan	MCL
	NCOA3	?/Taxol	Breast
miRNA-21	PDCD4	+/CDDP	Ovarian
	PTEN	+/Trastuzumab	Gastric
	FasL	+/GEM	Pancreas
	hMSH2	+/5-FU	Colorectal
miRNA-122	PKM2	?/5-FU	Colon
miRNA-133	ABCC1	?/DOX	HCC

include their ability to either promote or hinder cancer progression through sustaining proliferative signalling, resisting or promoting cell death, angiogenesis promotion, and promotion of drug resistance, miRNAs involved in drug resistance in various cancer tumours are discussed in Table-3. Since miRNAs expression regulate cancer at each stage, therefore, they are considered diagnostic biomarkers because if the miRNAs and their expression alterations can be detected through polymerase chain reaction (PCR), it can signal cancer or tumour occurrence and progression. The employment of miRNAs as diagnostic biomarkers is currently under consideration, as they have several advantages like being the least invasive strategy for the diagnosis with increased sensitivity. At the same time, however, they carry some disadvantages in terms of specificity and diversified origin.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Chen H, Liu H, Zou H, Chen R, Dou Y, Sheng S, et al. Evaluation of Plasma miR-21 and miR-152 as Diagnostic Biomarkers for Common Types of Human Cancers. J Cancer 2016;7:490-9. doi: 10.7150/jca.12351.
2. Abels ER, Breakefield XO. Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. Cell Mol Neurobiol 2016;36:301-12. doi: 10.1007/s10571-016-0366-z.
3. de la Torre Gomez C, Goreham RV, Bech Serra JJ, Nann T, Kussmann M. "Exosomics"-A Review of Biophysics, Biology and Biochemistry of Exosomes With a Focus on Human Breast Milk. Front Genet 2018;9:e92. doi: 10.3389/fgene.2018.00092.
4. Harding C, Heuser J, Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. J Cell Biol 1983;97:329-39. doi: 10.1083/jcb.97.2.329.
5. Matsumura T, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, Sawada G, et al. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. Br J Cancer 2015;113:275-81. doi: 10.1038/bjc.2015.201.
6. Bhome R, Del Vecchio F, Lee GH, Bullock MD, Primrose JN, Sayan AE, et al. Exosomal microRNAs (exomiRs): Small molecules with a big role in cancer. Cancer Lett 2018;420:228-35. doi: 10.1016/j.canlet.2018.02.002.
7. Tsang J, Zhu J, van Oudenaarden A. MicroRNA-mediated feedback and feedforward loops are recurrent network motifs in mammals. Mol Cell 2007;26:753-67. doi: 10.1016/j.molcel.2007.05.018.
8. Vidigal JA, Ventura A. The biological functions of miRNAs: lessons from in vivo studies. Trends Cell Biol 2015;25:137-47. doi: 10.1016/j.tcb.2014.11.004.
9. Peng Y, Croce CM. The role of MicroRNAs in human cancer. Signal Transduct Target Ther 2016;1:e15004. doi: 10.1038/sigtrans.2015.4.
10. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 2006;6:259-69. doi: 10.1038/nrc1840.
11. Sheu-Gruttadauria J, MacRae IJ. Structural Foundations of RNA Silencing by Argonaute. J Mol Biol 2017;429:2619-39. doi: 10.1016/j.jmb.2017.07.018.
12. Karginov FV, Cheloufi S, Chong MM, Stark A, Smith AD, Hannon GJ. Diverse endonucleolytic cleavage sites in the mammalian transcriptome depend upon microRNAs, Drosha, and additional nucleases. Mol Cell 2010;38:781-8. doi: 10.1016/j.molcel.2010.06.001.
13. Wong N, Wang X. miRDB: an online resource for microRNA target prediction and functional annotations. Nucleic Acids Res 2015;43:146-52. doi: 10.1093/nar/gku1104.
14. Schirle NT, Sheu-Gruttadauria J, Chandradoss SD, Joo C, MacRae IJ. Water-mediated recognition of t1-adenosine anchors Argonaute2 to microRNA targets. Elife 2015;4:e07646. doi: 10.7554/eLife.07646.
15. Uhlmann S, Mannsperger H, Zhang JD, Horvat EÁ, Schmidt C, Küblbeck M, et al. Global microRNA level regulation of EGFR-driven cell-cycle protein network in breast cancer. Mol Syst Biol 2012;8:570. doi: 10.1038/msb.2011.100.
16. Flamand MN, Gan HH, Mayya VK, Gunsalus KC, Duchaine TF. A non-canonical site reveals the cooperative mechanisms of microRNA-mediated silencing. Nucleic Acids Res 2017;45:7212-25. doi: 10.1093/nar/gkx340
17. Peng Y, Dai Y, Hitchcock C, Yang X, Kassis ES, Liu L, et al. Insulin growth factor signaling is regulated by microRNA-486, an underexpressed microRNA in lung cancer. Proc Natl Acad Sci U S A 2013;110:15043-8. doi: 10.1073/pnas.1307107110.
18. Li C, Hashimi SM, Good DA, Cao S, Duan W, Plummer PN, et al. Apoptosis and microRNA aberrations in cancer. Clin Exp Pharmacol Physiol 2012;39:739-46. doi: 10.1111/j.1440-1681.2012.05700.x.
19. Pichiorri F, Suh SS, Rocci A, De Luca L, Taccioli C, Santhanam R, et al. Downregulation of p53-inducible microRNAs 192, 194, and 215 impairs the p53/MDM2 autoregulatory loop in multiple myeloma development. Cancer Cell 2010;18:367-81. doi: 10.1016/j.ccr.2010.09.005.
20. Chen B, Xia Z, Deng YN, Yang Y, Zhang P, Zhu H, et al. Emerging microRNA biomarkers for colorectal cancer diagnosis and prognosis. Open Biol 2019;9:180212. doi: 10.1098/rsob.180212.
21. Bracken CP, Scott HS, Goodall GJ. A network-biology perspective of microRNA function and dysfunction in cancer. Nat Rev Genet 2016;17:719-32. doi: 10.1038/nrg.2016.134.
22. Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. Nat Rev Cancer 2015;15:321-33. doi: 10.1038/nrc3932.
23. Shen J, Stass SA, Jiang F. MicroRNAs as potential biomarkers in human solid tumors. Cancer Lett 2013;329:125-36. doi: 10.1016/j.canlet.2012.11.001.
24. Vanni I, Alama A, Grossi F, Dal Bello MG, Coco S. Exosomes: a new horizon in lung cancer. Drug Discov Today 2017;22:927-36. doi: 10.1016/j.drudis.2017.03.004.
25. Yan K, Gao J, Yang T, Ma Q, Qiu X, Fan Q, et al. MicroRNA-34a inhibits the proliferation and metastasis of osteosarcoma cells both in vitro and in vivo. PLoS One 2012;7:e33778. doi: 10.1371/journal.pone.0033778.
26. Walayat A, Yang M, Xiao D. Therapeutic Implication of miRNA in Human Disease. In: Sharad S, Kapur S, eds. Antisense Therapy. London, UK: IntechOpen Limited, 2018. DOI: 10.5772/intechopen.82738.
27. Hartig SM, Hamilton MP, Bader DA, McGuire SE. The miRNA Interactome in Metabolic Homeostasis. Trends Endocrinol Metab 2015;26:733-45. doi: 10.1016/j.tem.2015.09.006.
28. Si W, Shen J, Zheng H, Fan W. The role and mechanisms of action of microRNAs in cancer drug resistance. Clin Epigenetics 2019;11:25. doi: 10.1186/s13148-018-0587-8.