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Micro RNAs: Mechanism, Applications and Future Prospects

Abstract

MicroRNA (miRNA) is a short (about 21 to 23 nucleotides), non-coding, highly evolutionarily conserved and single-stranded RNA molecule that regulate gene expression at the level of translation. miRNAs are partially complementary to one or more messenger RNA (mRNA) molecules and their main function is to down regulate gene expression in a variety of manners, including translational repression, mRNA cleavage and deadenylation. miRNAs are post-transcriptional regulators that bind to complementary sequences on target messenger RNA transcripts (mRNAs), usually resulting in translational repression and gene silencing. So, these are involved in various types of diseases. The miRBase (hosted by the Sanger Institute) is the central online repository for miRNA nomenclature, sequence data, annotation and target prediction.

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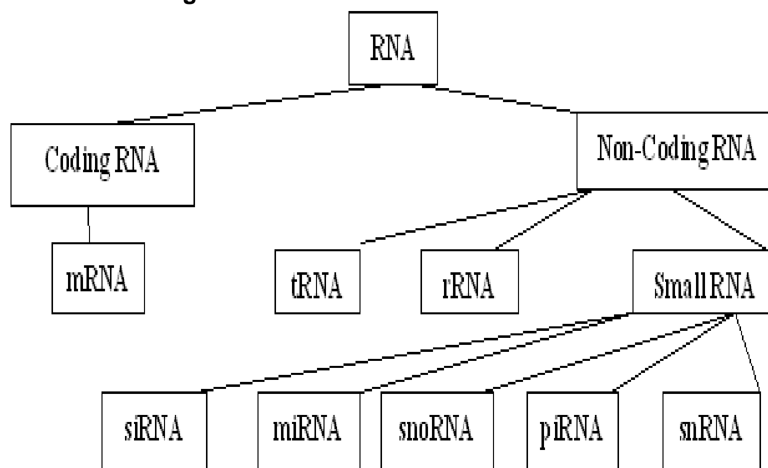
Introduction

RNA molecules that are not translate into a protein known as non-coding RNA (ncRNA). Due to the small size, it is also known as small RNA (sRNA). However, some ncRNA are very large (Xist). ncRNA are also known as, non-messenger RNA (nmRNA) or small non-messenger RNA (snmRNA).The DNA sequence from which a non-coding RNA is transcribed, the end product of this called non-coding RNA gene. Non-coding RNA genes including transfer RNA (tRNA), ribosomal RNA (rRNA), small RNAs such as small nucleolar RNA (snoRNA), small nuclear ribonucleic acid (snRNA), micro RNA (miRNA), small/ short interfering RNA (siRNA) and piwi-interacting RNA (piRNA) and long non-coding RNA (ncRNA). Figure 1 represents the types of various small RNA's.

Figure 1 Different classes of small RNAs



Aim of the Study

1. To explain about different types of small RNAs and MicroRNA.
2. To study the biogenesis of miRNA.
3. To know the regulatory functions of miRNAs.
4. To discuss the regulatory role of miRNA in various diseases.
5. To focus on the future aspects of miRNA as a biomarker, medicine and health care applications.

MicroRNAs (miRNAs) are small, non-coding, short ribonucleic acid (RNA) molecules. These are found in all eukaryotic cells, except fungi, algae and marine plants however miRNAs show very different characteristics between plants and animals (Table 1).

Table 1: miRNA's in Plants and Animals

Plants	Animals
miRNA complementarity to its mRNA target is nearly perfect, with no or few mismatched bases	miRNA complementarity is far from perfect and one miRNA can target many different sites on the same mRNA or on many different mRNAs.
In plants, targets can be located in the 3' UTR but are more often in the region itself	In metazoans, the miRNA target sites are in the three prime untranslated regions (3'UTR) of the mRNA

*(**Source:** He and Hannon 2004)*

The human genome contains about 1,000 miRNAs (Bentwich et al., 2005).These non-coding RNA molecules regulate the gene expression at transcriptional and translational level (Bartel, 2004; Fabbri et al., 2008; Bartel, 2009)). miRNAs are well conserved in eukaryotic organisms and are thought to be a vital and evolutionarily ancient component of genetic regulation (Tanzer and Stadler, 2004; Lee et al., 2007; Molnar et al., 2007; Kren et al., 2009). These can modulate vital processes e.g. cell cycle, metabolism, differentiation and development, tissue patterning and aging. Genes have been found in bacteria that are similar in the sense that they control mRNA abundance or translation by binding an mRNA by base pairing, however they are not generally considered to be miRNAs because the Dicer enzyme is not involved.

miRNAs were discovered in 1993 by Victor Ambros, Rosalind Lee and Rhonda Feinbaum during a study of the gene *lin-14* in worm *Caenorhabditis elegans* development (Lee et al., 1993) . They found that LIN-14 protein abundance was regulated by a short RNA product encoded by the *lin-4* gene. A 61-nucleotide precursor from the *lin-4* gene matured to a 22 nucleotide RNA that contained sequences partially complementary to multiple sequences in the 3′ UTR of the *lin-14* mRNA. This complementarity was both necessary and sufficient to inhibit the translation of the *lin-14* mRNA into the LIN-14 protein.

The second RNA was characterized in 2000 namely let-7, which repressed *lin-41*, *lin-14*, *lin-28*, *lin-42* and *daf-12* expression during developmental stage transitions in *Caenorhabditis elegans*. *let-7* was soon found to be conserved in many species (Pasquinelli, 2000; Reinhart et al., 2000), indicating the existence of a wider phenomenon. As of 2002, miRNAs have been confirmed in various animals and plants, including *Caenorhabditis elegans*, human and the plant *Arabidopsis thaliana*.

These are related to siRNA. miRNAs and siRNA also function similarly (Doench et al., 2003; Zeng et al., 2003; Filipowicz, et al., 2005) The siRNA technology has been studied for use in therapy prior to miRNA. miRNAs, like siRNAs, are expected to have low stability *in vivo* since they can be degraded by endogenous RNases. In addition, they can be fast eliminated by kidney filtration due to their small molecular mass. Although miRNA and siRNA both have gene regulation functions, there are subtle differences. Both miRNA and siRNA act on RNA interference (RNAi), but siRNA which is double stranded is best utilized for the cleaving of RNA when incorporated with RNA induced silencing complex (RISC) (Table 2).

Table 2: Role of miRNA and siRNA

miRNA	siRNA
miRNA is micro ribonucleic acid	siRNA is small interfering ribonucleic acid
miRNA is a natural molecule	siRNA is either a natural or a synthetic molecule
miRNA binds with its target imperfectly at several sites	siRNA binds with its target perfectly at a single site
miRNA plays an important role in gene regulation	siRNA has important functions in gene silencing
miRNA is single-stranded	siRNA is formed from two complementary strands. The two kinds of RNA are encoded slightly differently in the genome and the mechanism by which they regulate genes is slightly different
miRNA attaches to a piece of messenger RNA (mRNA) – which is the master template for building a protein – in a non-coding part at one end of the molecule. This acts as a signal to prevent translation of the mRNA into a protein.	siRNA, on the other hand, attaches to a coding region of mRNA, and so it physically blocks translation
miRNAs, as regulators of endogenous genes	siRNAs, as defenders of genome integrity in response to foreign or invasive nucleic acids such as viruses, transposons, and transgenes

Biogenesis of miRNA

It is proposed that miRNA may be synthesized either intergenic, intragenic or in exons (Rodriguez et al., 2004) but most miRNA genes are found in intergenic regions. Maximum miRNA (40%) genes may lie in the introns (intragenic)(Lau et al., 2001). The genes encoding these are much longer than the mature miRNA molecule. This DNA sequence includes the miRNA sequence and an approximate reverse complement. mi RNA may contain own promoter and regulatory units or may share with mRNA.(Lau et al., 2001; Lagos-Quintana, 2001; Lee and Ambros, 2001; Lee et al., 2004). miRNA genes that are transcribed from their own promoters, few primary transcripts have been fully identified. Many miRNAs are known to reside in introns of their pre-mRNA host genes and share their regulatory elements. miRNAs (42-48%) originating from poly-cistronic units contains 2-7 discrete loops from which mature miRNAs are processed (Lee et al., 2004; Altuvia et al., 2004). Although this does not necessarily mean the mature miRNAs of a family is

homologous in structure and function.These may be occur in sense or antisense orientation to gene but usually found in a sense orientation (Chai et al., 2004; Webber, 2005) and usually regulated together with their host genes (Altuvia et al., 2004; Baskerville and Bartel, 2005; Rodriguez et al., 2005).

Events in Nucleus

Formation of Primary miRNA (Pri-miRNA)

miRNA genes are usually transcribed by RNA polymerase II (Lee et al., 2004; Zhou et al., 2007). The polymerase binds to a promoter region of DNA which is found near the DNA sequence and later it forms the hairpin loop of the pre-miRNA. When this DNA sequence is transcribed into a single-stranded RNA molecule, the miRNA sequence and its reverse-complement base pair to form a double stranded RNA hairpin loop. The resulting transcript is capped with a specially modified nucleotide at the 5′ end and polyadenylated with multiple adenosines (a poly (A) tail), (Lee et al., 2004; Cai et al., 2004). This transcript is called primary miRNA (pri-miRNA). miRNAs are transcribed by RNA polymerase II are large RNA precursors. These precursors may be 100 to 1000 of nucleotides in length and contain one or more miRNA stem-loops (Lee et al., 2004; Cai et al., 2004). If a stem-loop precursor is found at the 3′ UTR, transcript may serve as a pri-miRNA and a mRNA (Cai et al., 2004). RNA polymerase III (Pol III) transcribes some miRNAs (those have upstream Alu sequences), tRNAs and mammalian wide interspersed repeat (MWIR) promoter units (Faller and Guo, 2008). But these promoters shown to have some similarities in their motifs to promoters of other genes transcribed by RNA polymerase II such as protein coding genes (Lee et al., 2004; Zhou et al., 2007).

miRNAs are transcribed as long RNA precursors (pri-miRNAs). A single pri-miRNA may contain from one to six miRNA precursors. Mature miRNA is not same as the DNA template. It goes for RNA editing and site-specific modification to yield differ products from those encoded by their DNA. Perhaps 16% of pri-miRNAs may be altered through nuclear RNA editing (Ohman et al., 2007; Kawahara, 2008; Winter et al., 2009). This increases the diversity and scope of miRNA. Most commonly, enzymes known as Adenosine Deaminases Acting on RNA (ADARs) catalyze adenosine to inosine (A to I) transitions. RNA editing can halt nuclear processing and alter downstream processes including cytoplasmic miRNA processing and target specificity (Kawahara et al., 2008).

Formation of pre-miRNA

The pri-miRNAs are processed in the nucleus by the microprocessor complex. It consists of the RNase III enzyme Drosha4 and the double-stranded RNA binding protein (Pasha/DGCR8). The resulting pre-miRNAs are approximately 70 nucleotides in length and are folded into imperfect stem loop structures. Each hairpin is flanked by sequences necessary for efficient processing. The double stranded RNA structure of the hairpins in a pri-miRNA is recognized by a nuclear protein known as DiGeorge Syndrome Critical Region 8 (DGCR8 or Pasha in invertebrates), DGCR8 associates with the enzyme Drosha. This is a protein that cuts RNA, to form the microprocessor complex (Gregory et al., 2006). In this complex, DGCR8 orients the catalytic RNase III domain of Drosha to liberate hairpins from pri-miRNAs by cleaving RNA about 11 nucleotides from the hairpin base (two helical RNA turns into the stem). The resulting hairpin structure is known as a pre-miRNA (precursor-miRNA). Pre-miRNA has a two-nucleotide overhang at its 3′ end. It is spliced directly out of introns, bypassing the microprocessor complex, are known as Mirtrons. Originally thought to exist only in *Drosophila* and *C. elegans*, mirtrons have now been found in mammals (Berezikov et al., 2007).

Transportation of pre-miRNA from nucleus to cytoplasm

Pre-miRNA is exported from the nucleus to the cytoplasm. It is accomplished by nucleocytoplasmic shuttle Exportin-5 (Exp5) and Ran-GTP complex (Yi et al., 2003). Exportin-5 (a member of the *karyopherin* family) recognizes a two-nucleotide overhang left by the RNase III enzyme Drosha at the 3' end of the pre-miRNA hairpin. It is an energy-dependent process. In this process GTP is bound to the Ran protein (Murchison and Hannon, 2004). Ran (ras related nuclear protein) is a small GTP binding protein.This protein is essential for the translocation of RNA and proteins through the nuclear pore complex (Moore et al., 1993). The Ran GTPase binds to Exportin-5 and forms a nuclear heterotrimer with pre-miRNAs (Yi et al., 2003, Lund et al., 2004). After transportation of the pre-miRNA in the cytoplasm, it undergoes an additional processing step by the RNAse III enzyme Dicer9 and generated a double stranded approximately 22 nucleotides length of miRNA.

Events in cytoplasm

In the cytoplasm, the pre-miRNA hairpin is cleaved by the RNase III enzyme Dicer (Lund and Dahlberg, 2006). Dicers are large 200 kDa proteins containing an ATPase/RNA helicase, a DUF283 (Domain of unknown function) domain, a PAZ (Piwi, Argonaut and Zwille) domain which bind the characteristic two base 3' overhangs of mi and siRNA, two catalytic RNase III domains (RIIIa and RIIIb) and a C-terminal double-stranded RNA-binding domain (dsRBD). Dicer functions as a monomer and has a single processing center with intramolecular dimerization of the two RNase III domains. Each RNase domain independently cuts one RNA strand of the duplex to generate products with 2-nucleotides on 3' overhangs. In addition to excising miRNAs from pre-miRNAs, Dicer enzymes process dsRNA to siRNAs. After Dicer cleavage, the miRNA pathway is similar to the central steps of RNA interference (RNAi) in animals. In contrast to siRNAs, microRNAs can direct RISC to down-regulate gene expression by translational repression (based on lower complementarity between miRNA and mRNA), or function as siRNAs do and mediate mRNA cleavage. If the miRNA is perfectly or nearly complementary to its target, it can specifically cleave the target mRNA. Endogenously expressed miRNAs are usually imperfectly complementary to their target gene(s) and

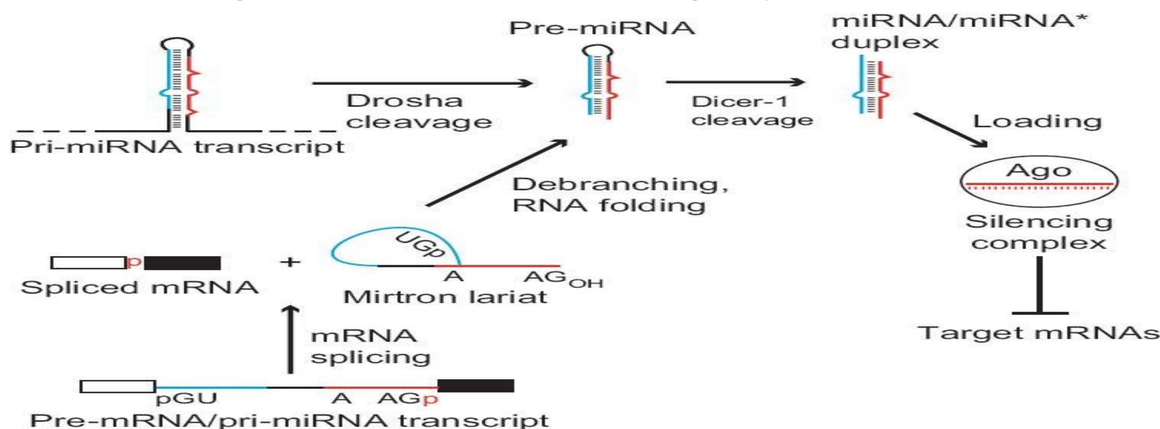
modulates the effect on gene expression via translational repression (Filipowicz et al., 2005).

This endoribonuclease interacts with the 3' end of the hairpin and cuts away the loop joining the 3' and 5' arms, yielding an imperfect miRNA: miRNA* duplex about 22 nucleotides in length (Lund E. and Dahlberg, 2006). Overall hairpin length and loop size influence the efficiency of Dicer processing and the imperfect nature of the miRNA: miRNA* pairing also affects cleavage (Lund and Dahlberg, 2006; Ji, 2008). Although either strand of the duplex may potentially act as a functional miRNA, only one strand is usually incorporated into the RNA-induced silencing complex (RISC) where the miRNA and its mRNA target interact.

Dicer also initiates the formation of the RNA-induced silencing complex (RISC) (Hammond, 2005). RISC is responsible for the gene silencing observed due to miRNA expression and RNA interference (Hammond et al., 2000).

The mature miRNA is part of an active RNA-induced silencing complex (RISC) containing Dicer and many associated proteins (Rana, 2007). RISC is also known as a microRNA ribonucleoprotein complex (miRNP); (Schwarz and Zamore, 2002) RISC with incorporated miRNA is also referred to as miRISC. Dicer processing of the pre-miRNA is thought to be coupled with unwinding of the duplex. Generally, only one strand is incorporated into the miRISC, selected on the basis of its thermodynamic instability and weaker base-pairing relative to the other strand (Khvorova, 2003; Schwarz et al., 2003; Krol et al., 2004). The position of the stem-loop may also influence strand choice (Lin et al., 2003). The incorporated strand is known as the guide strand and is selected by the argonaute protein on the basis of the stability of the 5' end. The remaining strand, known as the passenger strand (*) is degraded as a RISC complex substrate. The strand with lower stability base pairing of the 2–4 nucleotode at the 5' end of the duplex preferentially associates with RISC and thus becomes the active miRNA (Schwarz et al, 2003). After integration into the active RISC complex, miRNAs exert their regulatory effects by binding to imperfect complementary sites within the 3' untranslated regions (UTRs) of their mRNA targets. The formation of the double-stranded RNA, resulting from the binding of the miRNA, leads to translational repression. In some cases, both strands of the duplex are viable and become functional miRNA that target different mRNA populations (Okamura et al., 2008) (Figure 2).

Figure 2: miRNA formation and Processing (Ruby et al., 2007)



Members of the argonaute (Ago) protein family are central to RISC function. Argonautes are needed for miRNA-induced silencing and contain two conserved RNA binding domains (i) a PAZ domain that can bind the single stranded 3' end of the mature miRNA and (ii) a PIWI domain that structurally resembles ribonuclease-H and functions to interact with the 5' end of the guide strand. They bind the mature miRNA and orient it for interaction with a target mRNA. Some argonautes, for example human Ago2, cleave target transcripts directly. Argonautes may also recruit additional proteins to achieve translational repression (Pratt and MacRae, 2009). The human genome encodes eight argonaute proteins divided by sequence similarities into two families: AGO (with four members present in all mammalian cells and called E1F2C/hAgo in humans), and PIWI (found in the germ line and hematopoietic stem cells) (Schwarz and Zamore, 2002; Pratt and MacRae, 2009).

Regulatory Role of miRNAs

Researcher sare trying to understand the scope and diversity and applications of these regulatory molecules.It targets on so many genes but largely targets are unknown. One to hundreds of target genes for a given miRNA has been estimated by predictions using various bioinformatics tools.

The role of miRNAs in higher eukaryotes is regulation of gene expression. These are found in different cell types and tissues (Lagos-Quintana et al., 2002). These are likely to be involved in most biologic processes (Brennechke et al., 2003; Chen et al., 2004; Poy et al., 2004; Wilfred, 2004; Lim et al., 2005; Cuellar and McManus, 2005; Harfe, 2005). These are important as transcription factors. miRNA has multiple roles in negative regulation (transcript degradation and sequestering, translational suppression) and possible involvement in positive regulation (transcriptional and translational activation).

Researches on miRNAs shows that these are key regulators in several process for examples cancer (Liuxi Chen et al., 2017), ageing, (Cary and Keisuke, 2017), Hematopoiesis and hematologic disease (Cary and Keisuke, 2017), Cardiac aging (Robin Verjans et al., 2017), Neurodegenerative Diseases (Sean Quinlan et al., 2017) cell proliferation and cell death (Brennecke, 2003), early development (Reinhart, 2000), cell differentiation (Dostie, 2003, Chen, 2003), apoptosis and fat metabolism (Xu, 2003), brain development (Krichevsky, 2003), chronic lymphocytic leukemia (Calin, 2004), colonic adenocarcinoma (Michael, 2003),Gastric Carcinogenesis (Jinha Hwang et al., 2018) Burkitt's Lymphoma (Metzler, 2004), human embryonic stem cells (Morin et al., 2008)liver diseases (Dan et al., 2018)and viral infection (Pfeffer, 2004) etc. Changes in the miRNA expression pattern play an important role in diseases.A unique patterns of expression of miRNAs can be used for cancer diagnosis. So, these can be utilized as diagnostic molecular biomarkers (Chen 2005; Calin and croce 2006; Esquela-Kerscher and Slack 2006; Fabbri et al., 2008).

Huang et al. (2011) reported several muscle-specific miRNAs, including miR-1/206, miR-133 and miR-208. These muscle-specific miRNAs are essential for normal myoblast differentiation and proliferation. They have also been implicated in various cardiac and skeletal muscular diseases. miRNA-based gene therapies hold great potential for the treatment of cardiac and skeletal muscle diseases. So, research is also carry on miRNA-based therapies (Li et al., 2009; Fasanaro et al., 2010) due to due to their small size (23-24 nucleotides in length), natural antisense interactors, miRNA expression profiles can be used to diagnose a disease state because deregulated miRNAs can initiate and develop the diseases. So, these are future for the practice of medicine and health care applications. (Waldman et al., 2007; Waldman and Terzic, 2007; 2008).

An important goal for miRNA-mediated gene silencing is improving in the stability of miRNAs. To enhance stability of miRNA we can do the chemical modifications and bio-conjugation with lipids and peptides. We also need in-depth understanding of miRNA cellular uptake, distribution and the biological activity at organ and cellular level and on whole body. Cell-membrane receptors, several targeting ligands and antibodies can be used to achieve cell-specific delivery.It is proved that miRNAs have so many regulatory functions related to cell growth, development and differentiation. These are also associated with breast, lung, colon and human B-cell lymphocytes cancer (Meltzer, 2005; Croce, 2008). It was observed miRNA expression in tumors was differ to normal tissues (Meltzer, 2005). So, these human miRNAs cab be utilized as biomarkers for cancer diagnostics. The role of miRNAs in heart disease (Van Rooij et al., 2007) is also observed. Different expression pattern of miRNA is also reported in cardiac diseases cells. So these are the mark that suggests miRNA research has great promise in development of many medical therapies of tomorrow.

References

1. *Altuvia Y, Landgraf P, Lithwick G, Elefant N, Pfeffer S, Aravin A, Brownstein MJ, Tuschl T, Margalit H (2005). Clustering and conservation patterns of human microRNAs. Nucleic Acids Res.33 (8): 2697–706.*
2. *Bartel DP (2004). "MicroRNAs: genomics, biogenesis, mechanism, and function". Cell 116 (2): 281–97.*
3. *Bartel DP (2009). MicroRNAs: target recognition and regulatory functions. Cell 136 (2): 215–233.*
4. *Baskerville S, Bartel DP (2005). Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. RNA 11 (3): 241–7.*
5. *Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, Sharon E, Spector Y, Bentwich Z (2005). Identification of hundreds of conserved and nonconserved human microRNAs. Nat. Genet. 37 (7): 766–70.*
6. *Berezikov E, Chung W, Willis J, Cuppen E, Lai E (2007). Mammalian mirtron genes. Molecular cell. 28 (2): 328–336.*
7. *Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM (2003). Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. Cell. 113 (1): 25–36.*
8. *Cai X, Hagedorn CH, Cullen BR (2004). Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA. 10 (12): 1957–66.*
9. *Calin GA, Croce CM (2006). MicroRNA signatures in human cancers. Nat Rev Cancer. 6: 857–866.*
10. *Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM (2004). Human miRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc. Natl. Acad. Sci. USA. 101: 2999-3004.*
11. *Cary N Weiss, Keisuke Ito(2017). A Macro View of MicroRNAs: The Discovery of MicroRNAs and Their Role in Hematopoiesis and Hematologic Disease. International Review of Cell and Molecular Biology. 334, 99-175.*
12. *Chen CZ (2005). MicroRNAs as oncogenes and tumor suppressors. N Engl J Med. 353: 1768–1771.*
13. *Chen CZ, Li L, Lodish HF, Bartel DP (2004). MicroRNAs modulate hematopoietic lineage differentiation. Science. 303(5654): 83–6.*
14. *Chen X (2003). A MicroRNA as a Translational Repressor of APETALA2 in Arabidopsis Flower Development. Science E-Pub.*
15. *Croce, CM (2008). Molecular origins of cancer: Oncogenes and Cancer. N. Engl. J. Med. 358, 502–511.*
16. *Cuellar TL, McManus MT (2005). MicroRNAs and endocrine biology. J. Endocrinol. 187 (3): 327–32.*
17. *Dan Ye, Tianbao Zhang, Guohua Lou, Yanning Liu(2018). Role of miR-223 in the pathophysiology of liver diseases. Experimental & Molecular Medicine. 50, Article number: 128.*

18. *Doench JG, Petersen CP, Sharp PA (2003). siRNAs can function as miRNAs. Genes Dev. 17, 438-442*
19. *Dostie J, Mourelatos Z, Yang M, Sharma A, Dreyfuss G (2003) Numerous microRNPs in neuronal cells containing novel microRNAs. RNA 9(2): 180-6.*
20. *Esquela-Kerscher A, Slack FJ (2006). Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer. 6: 259–269*
21. *Fabbri M, Croce CM, Calin GA (2008).* MicroRNAs*. Cancer J.2008; 14: 1–6.*
22. *Faller M, Guo F (2008). MicroRNA biogenesis: there's more than one way to skin a cat.. Biochimica et Biophysica Acta 1779(11): 663–667.*
23. *Fasanaro P, Greco S, Ivan M, Capogrossi M, Martelli F (2010). MicroRNA: emerging therapeutic targets in acute ischemic diseases. Pharmacology & therapeutics. 125 (1): 92–104.*
24. *Filipowicz W, Jaskiewicz L, Kolb FA (2005). Post-transcriptional gene silencing by siRNAs and miRNAs. Curr. Opin. Struct. Biol. 15, 331-341.*
25. *Gregory R, Chendrimada T, Shiekhattar R (2006). MicroRNA biogenesis: isolation and characterization of the microprocessor complex. Methods in molecular biology (Clifton, N.J.) 342: 33–47.*
26. *Hammond SM et al. (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature, 404,293–296.*
27. *Hammond, SM (2005). Dicing and slicing: The core machinery of the RNA interference pathway. FEBS Lett. 579, 5822–5829.*
28. *Harfe BD, McManus MT, Mansfield JH, Hornstein E, Tabin CJ (2005). The RNaseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb. Proc. Natl. Acad. Sci. U.S.A. 102 (31): 10898–903.*
29. *He L, Hannon GJ (2004). MicroRNAs: small RNAs with a big role in gene regulation. Nat. Rev. Genet. 5 (7): 522–31.*
30. *Huang ZP, Neppl RL Jr, Wang DZ(2011). Application of microRNA in cardiac and skeletal muscle disease gene therapy. Methods Mol Biol. 709:197-210.*
31. *Ji X (2008). The mechanism of RNase III action: how dicer dices. Current topics in microbiology and immunology 320: 99–116.*
32. *Jinha Hwang, Byung-Hoon Min, Jiryeon Jang, So Young Kang, Hyunsik Bae, Se Song Jang, Jong-I Kim, Kyoung-Mee Kim (2018). MicroRNA Expression Profiles in Gastric Carcinogenesis. Scientific Reports. 8, Article number: 14393*
33. *Kawahara Y, Megraw M, Kreider E, Iizasa H, Valente L, Hatzigeorgiou A, Nishikura K (2008). Frequency and fate of microRNA editing in human brain. Nucleic acids research. 36 (16): 5270–5280.*
34. *Khvorova A, Reynolds A, Jayasena SD (2003). Functional siRNAs and miRNAs exhibit strand bias. Cell. 115 (2): 209–16.*
35. *Kren BT, Wong PY, Sarver A, Zhang X, Zeng Y, Steer CJ (2009). MicroRNAs identified in highly purified liver-derived mitochondria may play a role in apoptosis. RNA Biol. 6 (1): 65–72.*
36. *Kreth, Simone, Hübner, Max, Hinske, Ludwig, Christian (2018). MicroRNAs as Clinical Biomarkers and Therapeutic Tools in Perioperative Medicine.Basic Science. 126 (2), 670–681.*
37. *Krichevsky AM, King KS, Donahue CP, Khrapko K, Kosik KS (2003). A miRNA array reveals extensive regulation of miRNAs during brain development. RNA. 9: 1274-1281.*
38. *Krol J, Sobczak K, Wilczynska U, Drath M, Jasinska A, Kaczynska D, Krzyzosiak WJ (2004). Structural features of microRNA (miRNA) precursors and their relevance to miRNA biogenesis and small interfering RNA/short hairpin RNA design. J Biol Chem. 279 (40): 42230–9.*
39. *Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T (2001). Identification of novel genes coding for small expressed RNAs. Science. 294 (5543): 853–8.*
40. *Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T (2002). Identification of tissue-specific microRNAs from mouse. Curr. Biol. 12 (9): 735–9.*
41. *Lau NC, Lim LP, Weinstein EG, Bartel DP (2001). An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science. 294 (5543): 858–62.*
42. *Lee CT, Risom T, Strauss WM (2007). Evolutionary conservation of microRNA regulatory circuits: an examination of microRNA gene complexity and conserved microRNA-target interactions through metazoan phylogeny. DNA Cell Biol. 26 (4): 209–18.*
43. *Lee RC, Ambros V (2001). An extensive class of small RNAs in Caenorhabditis elegans. Science. 294 (5543): 862–4.*
44. *Lee RC, Feinbaum RL, Ambros V (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 75 (5): 843–54.*
45. *Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN (2004). MicroRNA genes are transcribed by RNA polymerase II". EMBO J. 23 (20): 4051–60.*
46. *Li C, Feng Y, Coukos G, Zhang L (2009). Therapeutic microRNA strategies in human cancer. The AAPS journal. 11 (4): 747–57.*
47. *Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM (2005). Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature. 433 (7027): 769–73.*
48. *Lin SL, Chang D, Ying SY (2005). Asymmetry of intronic pre-miRNA structures in functional RISC assembly. Gene. 356: 32–8.*
49. *Liuxi C, Yuxia Z, Qiuhua S, Jichun Z, Hongming P, Xinbing S (2017). Regulation of Autophagy by MiRNAs and Their Emerging Roles in Tumorigenesis and Cancer Treatment. International Review of Cell and Molecular Biology, 334, 2017, 1-26.*

50. *Lund E et al. (2004). Nuclear export of microRNA precursors. Science, 30, 95–98.*
51. *Lund E, Dahlberg J, (2006). Substrate selectivity of exportin 5 and Dicer in the biogenesis of microRNAs. Cold Spring Harbor symposia on quantitative biology 71: 59–66.*
52. *Mathieu N, Florence C, Éric B, Vincent DG, Frédérick A (2017). MalletteMolecular Regulation of Cellular Senescence by MicroRNAs: Implications in Cancer and Age-Related DiseasesPages 27-98*
53. *Meltzer PS (2005). Cancer genomics: Small RNAs with big impacts. Nature, 435, 745–746.*
54. *Metzler M, Wilda M, Busch K, Viehmann S, Borkhardt A (2004). High expression of precursor miRNA-155/BIC RNA in children with Burkitt lymphoma. Genes Chromosomes Cancer. 2: 167-169.*
55. *Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP, James RJ (2003). Reduced accumulation of specific microRNAs in colorectal neoplasia. Molecular Cancer Research. 1: 882-91.*
56. *Molnár A, Schwach F, Studholme DJ, Thuenemann EC, Baulcombe DC (2007). miRNAs control gene expression in the single-cell alga Chlamydomonas reinhardtii. Nature 447(7148): 1126–9.*
57. *Moore MS et al. (1993). The GTP-binding protein Ran/TC4 is required for protein import into the nucleus. Nature. 365, 661–663.*
58. *Morin et al(2008). Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells. Genome Res. 18(4):610-21.*
59. *Murchison E, Hannon G (2004). MiRNAs on the move: miRNA biogenesis and the RNAi machinery. Current opinion in cell biology. 16 (3): 223–229.*
60. *Ohman M (2007). A-to-I editing challenger or ally to the microRNA process. Biochimie 89 (10): 1171–1176.*
61. *Okamura K, Chung WJ, Lai EC (2008). The long and short of inverted repeat genes in animals: microRNAs, mirtrons and hairpin RNAs. Cell Cycle. 7 (18): 2840–5.*
62. *Pasquinelli et al (2000). Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature.408 (6808): 86–9.*
63. *Pfeffer S, Zavolan M, Grasser FA, Chien M, Russo JJ, Ju J, John B, Enright AJ, Marks D, Sander C, Tuschl T (2004). Identification of virus-encoded microRNAs. Science. 304(5671): 734-6.*
64. *Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P, Stoffel M (2004). A pancreatic islet-specific microRNA regulates insulin secretion. Nature. 432 (7014): 226–30.*
65. *Pratt A, MacRae I (2009). The RNA-induced silencing complex: a versatile gene-silencing machine. The Journal of biological chemistry 284 (27): 17897–17901.*
66. *Rana TM (2007). Illuminating the silence: understanding the structure and function of small RNAs. Nature Reviews Molecular Cell Biology 8 (1): 23.*
67. *Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G (2000). The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature.403 (6772): 901–6.*
68. *Robin V,Marcvan B,Blanche S (2017).MiRNA Deregulation in Cardiac Aging and Associated Disorders. International Review of Cell and Molecular Biology.334, 207-26.*
69. *Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A (2004). Identification of mammalian microRNA host genes and transcription units. Genome Res. 14 (10A): 1902–10.*
70. *Ruby JG, Jan CH, Bartel DP (2007). Intronic microRNA precursors that bypass Drosha processing. Nature. 5, 448(7149):83-6.*
71. *Ryan D Morin et al., (2008). Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells. Genome Res. 2008 18: 610-621.*
72. *Schwarz D, Zamore P (2002). Why do miRNAs live in the miRNP? Genes & development. 16 (9): 1025–1031.*
73. *Schwarz DS, Hutvágner G, Du T, Xu Z, Aronin N, Zamore PD (2003). Asymmetry in the assembly of the RNAi enzyme complex. Cell. 115 (2): 199–208.*
74. *Sean Q, AidanK,Miguel M,'TobiasE,Eva M, Jimenez-M(2017). MicroRNAs in Neurodegenerative Diseases. International Review of Cell and Molecular Biology. 334, 309-343.*
75. *Tanzer A, Stadler PF (2004). Molecular evolution of a microRNA cluster. J. Mol. Biol. 339 (2): 327–35.*
76. *van Rooij, E. et al. (2007). MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets. J. Clin. Invest. 117, 2369–2376.*
77. *Waldman SA, Teizic A(2008).* MicroRNA signatures as diagnostic and therapeutic targets*. Clin Chem.54: 943–944.*
78. *Waldman SA, Teizic MR, Teizic (2007).* Molecular medicine hones therapeutic arts to science*. Clin. Pharmacol. Ther.2007; 82: 343–347.*
79. *Waldman SA, Terzic A (2007). Translating MicroRNA discovery into clinical biomarkers in cancer. JAMA. 297: 1923–1925.*
80. *Weber MJ (2005). New human and mouse microRNA genes found by homology search. FEBS J. 272 (1): 59–73.*
81. *Wilfred BR, Wang WX, Nelson PT (2007). Energizing miRNA research: a review of the role of miRNAs in lipid metabolism, with a prediction that miR-103/107 regulates human metabolic pathways. Mol. Genet. Metab. 91 (3): 209–17.*
82. *Winter J, Jung S, Keller, S, Gregory R, Diederichs S (2009). Many roads to maturity: microRNA biogenesis pathways and their regulation. Nature cell biology 11 (3): 228–234.*
83. *Xu P, Vernooy SY, Guo M, Hay BA. (2003) The Drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. Curr Biol 13(9): 790-5.*
84. *Yi R et al (2003). Exportin-5 mediates the nuclear export of pre-microRNAs and short Hairpin RNAs. Genes Dev. 17, 3011–3016.*
85. *Zeng Y, Yi R, Cullen BR et al(2003). MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proc. Natl Acad. Sci. USA 100, 9779-9784.*
86. *Zhou X, Ruan J, Wang G, Zhang W (2007). Characterization and identification of microRNA core promoters in four model species. PLoS Comput. Biol. 3 (3): e37.*