Animal Health Prod and Hyg (2021) 10 (2) : 58 - 65

Animal Health, Production and Hygiene

www.aduveterinaryjournal.com

Review

miRNA and Biogenesis

Beyza SUVARIKLI ALAN[1*], Mehmet NİZAMLIOĞLU[1], Zafer BULUT[1]

[1] *Selçuk University, Faculty of Veterinary Medicine, Department of Biochemistry, 42031 Konya, Turkey*

ABSTRACT

The number of uncoded (non-coding; nc) RNAs with known functions is increasing each day. Since their first detections in 1993, micro RNA of ncRNAs have become very important as shown by many studies. In fact, their importance has been well understood and their relations with diseases are well documented that they can be used in the diagnosis of most diseases in the future. In this review, topics such as general properties, structure and biogenesis of miRNAs were discussed.

Keywords: miRNA, Drosha, Dicer

miRNA ve Biyogenezi

ÖZET

Fonksiyonu belirlenen kodlanmamış (non-coding; nc) RNA'ların sayısı her geçen gün artmaktadır. İlk olarak 1993 yılında bulunmasından sonra çok sayıda araştırma ile ncRNA'lardan olan miRNA'lar çok önemli yere sahip olmuşturlar. Hatta önemi her geçen gün daha çok anlaşılmakta ve hastalıklar ile ilişkileri daha çok belirlenmekte, gelecekte de çoğu hastalığın teşhisinde kullanılabilecekleri açıktır. Bu derlemede, miRNA'ların genel özellikleri, yapısı, biyogenezi gibi konular ele alınmıştır.

Anahtar kelimeler: miRNA, Drosha, Dicer

Corresponding Author: Beyza SUVARIKLI ALAN, Selçuk University, Faculty of Veterinary Medicine, Department of Biochemistry, 42031 Konya, Turkey e-mail: beyza.alan@selcuk.edu.tr
Received Date: 11.10.2021 – Accepted Date: 01.12.2021
DOI Number: 10.53913/aduveterinary.1008317

Introduction

Gene editing is focused on genes that encode proteins through DNA-mRNA-protein dogma. Cellular functions are regulated by the expression of protein encoding or non-coding (nc) RNAs that have important functions in both nucleus and cytoplasm (Williams et al., 2018). Therefore, it is important to know the RNA structure and to understand the functions of all RNAs. In addition, RNAs are also critical because of their role in the regulation of gene expression (Mortimer et al., 2014). RNA is a biopolymer consisting of organic bases (adenine, uracil, guanine, cytosine), ribosine sugar, and a phosphate group. These monomers combine between the 3′ end of one sugar molecule and 5' carbon atom of the other by having a phosphodiester bond. All prokaryote and eukaryote cells have three main types of RNA such as messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA) (Jedrzejczyk et al., 2017). A wide range of small RNAs have been found and recent developments have led to the discovery of small RNAs in eukaryotic cells. Noncoding RNAs from these RNAs have been examined in three different categories based on biogenesis, their mechanism and the type of Argounaute protein they interact. Of these, small noncoding RNAs are microRNAs (miRNA), small interfering RNAs (siRNA), and Piwi-interacting RNAs (piRNA) (Kim et al., 2009). The discovery of miRNAs, the subject of this review, is very important in modern molecular biology and genetics. A large number of cell pathways, which are important in both disease and health condition, appear to be regulated by miRNAs (Shukla et al., 2011). The number of miRNAs defined by the development and use of highly efficient sequencing techniques is abundant these days (Olena and Patton, 2010). miRNAs, which target most protein-coding transcripts, play a role in almost all developmental and pathological events in eukaryotes. It is reportedly associated with many human diseases, including cancer and neurodevelopmental disorders (Ha and Kim, 2014).

Classification of Non-Coding RNAs

ncRNAs are usually distinguished by their length; Short non-coding RNAs have a length of <30 nucleotides while long non-coding RNAs have a nucleotide count of more than 200. In addition, these RNAs are molecules acting as negative regulators of gene expression in the RNA interference (RNAi) mechanism (Santosh et al., 2014).

Short non-coding RNAs are divided into three main groups such as miRNA, piRNA, and siRNA. These RNAs are quite difficult to distinguish from each other (Carthew and Sontheimer, 2009). Obviously, biochemically and functionally, miRNA and siRNA are indistinguishable. These molecules can be distinguished by their origin.

When these three RNA types are examined in terms of common and different characteristics:

1-While siRNA and miRNA bind to argonoute proteins, piRNAs bind to Piwi members (Carthew and Sontheimer, 2009).

2- miRNAs and siRNAs are both approximately 19-20 nucleotide lengths.

3- They combine with the RISC complex in silencing gene expression (MacFarlane and Murphy, 2010).

4- The cellular origins of miRNA and siRNA are slightly different; miRNAs are endogenous products of an organism's genome while siRNAs are mostly caused by exogens derived from viruses and similar organisms (Carthew and Sontheimer, 2009; Wilson and Doudna, 2013).

5- siRNAs are complementary to their targets whereas miRNAs (those found in animals) demonstrate limited complementariness (Shabalina and Koonin, 2008).

6- miRNA sequences are almost always preserved in the relevant organism whereas siRNA sequences are rarely preserved (Bartel, 2004).

7- siRNAs are found in plants, animals and fungi. It provides antiviral defense in plants and animals. By contrast, miRNAs are found only in terrestrial plants, single-celled green *algae Chlamydomonas reinhardtii* and metazoan animals. They are not found in single cell choanoflagellates and fungi (Ghildiyal and Zamore, 2009).

History of miRNA

In 1993, Lee and his *colleagues* first discovered a miRNA called linage-4 (lin-4), which was 22 nt (nucleotide) long and did not encode a protein in *Caenorhabditis elegans*. In the study conducted by Wightman et al. (1993) on C. elegans, it was shown that lin-4 acts as a negative regulator of the lin-14 gene. Reinhart et al. (2000) discovered a miRNA, called lethal-7 (let-7), a 22 nt long in *C. elegans* that has been shown to control the transition from larvae to adulthood. It was observed that transition from the larval period to the adult period was impired when there was a loss in the activation of Let-7. After the discovery of Let-7, the short RNA's were called micro RNA (miRNA) (Lucas and Raikhel, 2013; Bartel, 2004; Wienholds and Plasterk, 2005; Carthew and Sontheimer, 2009; Tetreault and Guire, 2013).

Small RNAs do not encode any proteins, but rather terminate the functions of protein-coding mRNAs. Due to these functions, they are known as the negative control mechanism of gene expression (Carthew and Sontheimer, 2009). These RNA molecules are present in precursor forms with no functions when they are first produced. They are then processed and acquire their functional structures (Kim et al., 2009). First discovered in *Caenorhabditis elegans*, miRNAs were later shown to exist in many eukaryotes, including humans (MacFarlane and Murphy, 2010). Complex genomes are known to encode hundreds of miRNA genes (Graves and Zeng, 2012). It is thought that about 1000 miRNAs in the human genome are encoded (Cowland et al., 2007). Most miRNAs are expressed from genome regions that

differ from known protein encoding sequences (Du and Zamore, 2005). In other words, most miRNAs are produced within the cell by non-encoding gene regions (Cai et al., 2004). The coding loci of some miRNAs are located separately from other miRNAs and indicate that they create their own transcription units; others cluster and share similar patterns of expression, which indicates that they are transcribed as polycistronic transcripts (Du and Zamore, 2005). About half of the known mammalian miRNAs are found in introns of protein-coding genes or introns or exons of non-encoding RNAs (Du and Zamore, 2005).

Biogenesis and processing of Non-Coding RNAs

The biogenesis of miRNAs is a highly complex, tightly regulated multi-step transcription that begins in the nucleus of the cell and continues throughout the cytoplasm through which mature miRNA completes its main function (Melo and Melo, 2013). Since miRNA biogenesis contains multiple steps, the abnormalities that occurs at any step of the process can prevent effective miRNA maturation (Williams et al., 2018). miRNAs are non-encoded RNAs, which means that they are transcribed but do not yield protein synthesis. However, this does not imply that these RNAs do not contain information and have no functions (Mattick and Makunin, 2006). Mature miRNA molecules are thought to be partially complementary with one or more mRNA. Their main function is to regulate downstream gene expression (Pillai, 2005).

miRNAs are produced with the action of two RNase-type proteins (Dicer and Drosha). Then they are connected with Ago proteins, the main component of the RISC complex. In this way, they perform their functions as post-transcription regulators (Kim et al., 2009). In miRNA biogenesis, miRNAs are first copied to the primary miRNA (pri-miRNA). These pri-miRNAs have a 7-methylguanosine cap at the 5' end, such as mRNAs. At the 3' end is the poly A tail (MacFarlane and Murphy, 2010). pri-miRNAs consist of a single chain. They curl up on themselves to form hairpin structures (Melo and Melo, 2013). Then, the pri-miRNA is cleaved by the enzyme Drosha in the nucleus. That is, they cleave the hairpin structure of the pri-miRNA. Enzyme-cut miRNAs turn into pre-miRNA (Alvarez-Garcia and Miska, 2005). Exportin-5 (EXP-5) in the nucleus mediate the transport of pre-miRNAs to the cytoplasm. Exportin-5 (EXP5) is a Ran-GTP-bound nuclear transport receptor (Wahid et al., 2010). They mediate the transfer of Ran-GTP RNA from the cell nucleus (Lei and Silver, 2002). EXP5, which binds to pre-miRNA, allows pre-miRNA to be removed from the nucleus as a result of the hydrolysis of GTP (with GDP formation). pre-miRNAs in the cytoplasm are processed by Dicer (protein) to become mature miRNAs (Wahid et al., 2010).

During the encounter of the miRNA and mRNA target sequences, it was found that the 2-8-based nucleotides of miRNA were bound to an excellent complementary recognition sequence on Mrna. Located at the 5' end of miRNAs, complementary 2-8 basis to the 3' UTR (untranslated region) region of the target mRNA is also called the seed region (Jansson and Lund, 2012). The comprehensive match between the miRNA seed region and mRNA is one of the main criteria used to identify mRNA targets (Olena and Patton, 2010). The central part (typically 10-11 nt) of miRNA usually lacks complementariness to mRNA. However, the 3'-region of miRNA is more or less specifically connected to mRNA and partly contributes to the specificity and efficacy of the miRNA:mRNA complex (Jansson and Lund, 2012). It has been discovered that most of these miRNAs are located in intergenic regions and some in intronic regions (Wahid et al., 2010).

The main component of the RISC (RNA-induced silencing complex) complex is argonaute proteins. The RISC complex ensures the selection and removal of the RNA strand with the lowest thermodynamic stability at the 5' end (Tetreault and De Guire, 2013). A strand of double-strand miRNAs binds to the Argonuate-2 (Ago-2) connected to the RISC complex, binding to the promoter region, 3'UTR, and 5'UTR of the target mRNA. Then they act as the regulator of gene expression (Melo and Melo, 2013; Bartel, 2009). This is done in two ways: the first is the inhibition of the initiation of translation, and the second is the degradation of mRNA (Betel et al., 2007).

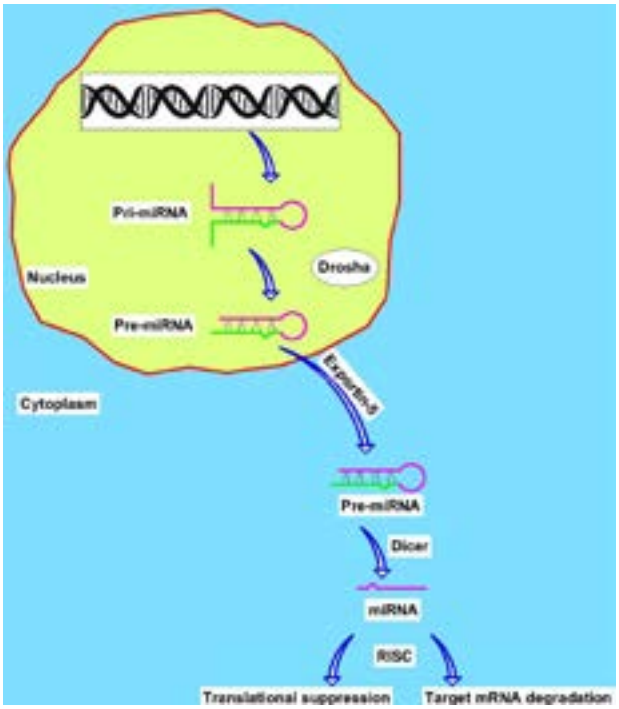

Figure 1. General microRNA pathway

miRNA: microRNA, Drosha: RNAse III enzyme, RISC: RNA-induced silencing complex, primiRNA: primary microRNA.

Structure and Properties of miRNAs

miRNAs play a crucial role in post-transcription gene

regulation in mammals through mRNA degradation and translational suppression (Williams et al., 2018). miRNAs are small, regulatory, and non-encoding RNA molecules. In general, they control the expression of the target mRNA through 3'UTR binding (Shukla et al., 2011). 3-UTR of the RNA transcripts located between the protein coding region of mRNA and the poly (A) tail (Jansson and Lund, 2012). miRNAs are non-encoded RNAs approximately 22 nt long and have their own hairpin structure. To create this, they are derived from self-folding transcripts (Bartel, 2009). miRNAs are initially copied into long chain structures, then go through stages to produce mature miRNAs (Graves and Zeng, 2012). A single miRNA can affect hundreds of targets, i.e. mRNA (Wienholds and Plasterk, 2005). In the earlier studies, the miRNA profile was performed on samples extracted from tissues, and subsequent studies found that there were miRNAs in bodily fluids such as serum, plasma, urine and saliva (MacFarlane and Murphy, 2010).

Naming and classifying of miRNAs

The first miRNAs discovered were named according to their phenotypes (let-7, lin-4 vb.) (Ha and Kim, 2014). Later, miRNAs are named by pre-appendix "mir " or "miR". Pre-mir refer to the pre-miRNA form while miR refer to the mature miRNA form (Hydbring and Badalian-Very, 2013). Under normal circumstances, pre-miRNAs and mature miRNAs are exactly the same.

Since they are located in other locations in the genome, pre-fix and numerical naming are also needed to indicate this. In addition, triple pre-fixes are used to indicate the type in which miRNA is present (e.g. rno-mir-34a rno=*Rattus norvegicus*). In the example of miR-15a and miR-15b; they both have the same 5' ends, but differ in four nucleotides in the 3' regions (Ambros et al., 2003; Hydbring and Badalian-Very, 2013).

UTR Structure

As well as the target sequence, the UTR structure is also important in connecting miRNA to the target mRNA (Ha and Kim, 2014). Mature and functional ncRNAs are connected to their target molecules, RNAs, from the 3'UTR region to control the expression of target mRNAs (Bartel, 2009). The most common method used to verify the target region of miRNA is to clone the predicted target mRNA's UTR to the luciferase reporter. By linking the target UTR to the lusiferase, it will demonstrate whether the change in lusiferase binds to UTR and regulates the expression of the gene at the mRNA or protein level (Cowland et al., 2007). The UTR region is known to have many functions in mRNA metabolism, such as transport, localization, translation efficiency, and stability (Krol et al., 2010).

Drosha

Since their first discovery in *Escherichia coli*, RIIID (RNase III domain) has been found in various proteins of different sizes from ~140 (mini-III) to ~1,900 (Dicer) amino acid residues. It is stated that proteins with RNAse activity are also generally protected in all bacteria and eukaryote species. The RNase III family have RNase III of bacterial origin and Rnt1p, Drosha, and Dicer of eukaryotic; among these *E. coli* RNase III is the most extensively investiaged member (Court et al., 2013). The bacterial RNAse III carries a single catalytic RNase III domain and a dsRNA binding domain (dsRBD) in terminal C. The enzyme drosha nuclease carries two catalytic RNase III domains and dsRNA binding domain (dsRBD) (Kwon et al., 2016).

It is known that the stages of microRNA maturation are initiated by Drosha, an RNase III (Kwon et al., 2016). This protein has about 160 kDa (Ha and Kim, 2014). A large protein, Drosha, has multiple domains and it increaes the overall production rate of miRNA by producing 3' protruding ends effectively recognized by Exp5 and Dicer (Han et al., 2004). Drosha is very similar in structure to Dicer, but compared to Dicer Drosha uses a rather complex mechanism. The structural comparison reveals some common characteristics between Drosha and Dicer. The first similarity is the length and conformation of the connector helix. Both Dicer and Drosha were found to share a proline, which causes bending in the binding helix. Despite this general similarity, there are very pronounced differences between Drosha and Dicer. Drosha is larger than Giardia Dicer and human Dicer, and contains an additional β sheet, at least one more α helix, and two ZnF motifs (Kwon et al., 2016).

Correct pri-miRNA process by Drosha requires dsRBD protein known as Pasha in Drosphila, *Pash-1 in C. elegans*, and DCGR8 in mammals (Du and Zamore, 2005*).* The DCGR8 gene was initially detected in the "DiGeorge syndrome chromosome region (DGCR)" of the human genome (Han et al., 2004).

Dicer

Dicer is a protein that has proven to exist in almost all eukaryotes (Vermeulen et al., 2005). This protein weighs about 200 kDa (Ha and Kim, 2014). It was called Dicer because it cleaves double-chain RNAs into small RNAs of equal size (Bernstein et al., 2001). Dicer was initially described in Drosophila and was later reported in humans, plants, and fungi (Singh et al., 2008). It was first recognized for its role in the production of small interfering RNAs that mediated the RNA interference (Bartel, 2004). It is a multi-domain enzyme of the RNase III family. It is stated that it plays a role in the degradation of double helix siRNA and miRNA (Singh et al., 2008). Dicer also initiates the formation of RISC which is complex for silencing of genes caused by miRNA expression and RNA interference (Betancur and Tomari, 2012). Although the Dicer protein differs in animal and plant species, it is characterized by the presence of N-terminal helicase domain, PAZ domain, dsRNA binding domain in C-terminal, and two catalytic RNAase III domains (MacRae and Doudna, 2007). The dicer enzyme C terminal, just like the Drosha protein, creates an intra-molecular dimer to form a catalytic center. The N-terminal helicase domain makes it easier

to recognize pre-miRNA and increases the functions of miRNAs (Ha and Kim, 2014). The presence of DUF 283 domain, whose function is still unclear, has also been shown. In addition, Dicer is a special ribonuclease which cleaves RNAi double-strand RNA (dsRNA) into small RNA fragments about 21-27 nt long (Tijsterman et al., 2004).

The Dicer's crystal structure was reported from the *G. intestinalis* parasite. Dicer of Giardia is smaller than eukaryotic Dicer enzymes because an amino-terminal helicase and carboxy-terminal dsRNA-binding domain (dsRBD) were found to be missing (Macrae et al., 2006). Giardia Dicer is a molecule of about 100 Å length and 30–50 Å width. Dicer recognizes the PAZ domain located at the end of a dsRNA. It then cleaves the dsRNA from RNase III domains. The distance between the PAZ and RNase III domains controls the length of small RNAs produced by Dicer. In Giardia dicer, this distance is about 65 Å and it generates small RRNAs with a length of 25 nt (Macrae et al., 2006). Paz in humans is a large protein containing dsRNA binding (dsRBD) and helicase fields, as well as two RNase III-like domain (Vermeulen et al., 2005).

RISC Complex and Ago Proteins

They use protein-RNA complexes called RISC for sequence-specific gene silencing through translational inhibition, mRNA destruction, and heterochromatin formation (Macrae et al., 2006). Depending on the complementarity of miRNA-mRNA, the RISC complex can be connected to 3'UTR portions of the target mRNA (Singh et al., 2008). Argonaute proteins function as the core components of RISC (Macrae et al., 2006) and various Argonaute proteins are isolated.

Argonaute proteins are proteins that exist in eukaryotes and prokaryotes. Ago proteins in eukaryotes are well known for their role in silencing RNA, while in prokaryotes their function is not fully known, but they are predicted to perform tasks similar to those in eukaryotes (Hall, 2005). Ago proteins are divided into 3 basic families: the first is known as AGO found in mammals, and the second is known as WAGO, which is found only in nematodes (worms). Ago proteins have four main domains: N-terminal, PAZ, Mid, and PIWI. In eukaryotes, in gene-regulating mechanisms mediated by small RNAs, AGO proteins are always divided into these areas (Hutvagner et al., 2008). The PAZ domain is defined as an area of 110 amino acids found in both dicer and ago proteins (Collins et al., 2005). Structural studies show that PAZ domain is connected to RNAs at the 5' end of the PIWI domain while PAZ domain is connected to 3' ends of single-strand RNAs, and PIWI structure is very similar to RNase H, and biochemical analyses indicate that AGO is the endonuclease of RISC (Du and Zamore, 2005). All hypotheses are based on the Drosophila model (MacFarlane and Murphy, 2010).

Ago2 is the only Argonaute with endonuclease activity in mammalian cells (Karginov et al., 2010). For example, dAgo1 and dAgo2 are found in separate complexes that contain siRNAs or miRNAs, respectively (Denli and Hannon, 2003). A study in human cells also demonstrated that AGO proteins bind to 3'UTR regions of mRNAs and stimulate translation, and it was suggested that these proteins are released during suppression and activation (Höck et al., 2008).

RNA Interference (RNAi)

The RNAi pathway was first demonstrated when trying to increase the expression of the chalcone synthase (CHS) gene in the Petunia plant. In this study, marbling petunia was obtained while trying to obtain dark purple petunia. As a result, it was stated that it can be a gene silencing mechanism following transcription (Napoli et al.,1990; Agrawal et al., 2003). It was later described as a response to long double-chain RNA (dsRNA) exogenously inserted to *C. elegans* (Fire et al., 1998; Doench et al., 2018). Fire and Mello (1998) won the Nobel Prize in 2006 for their work on RNAi, which silenced gene expression with siRNA and miRNA (Lucas and Raikhel, 2013). RNAi is a post-transcription gene silencing mechanism (Kim and Rossi, 2008) that causes the double-strand RNA to break down when it enters the cell. The key points of the RNAi mechanism are miRNAs and siRNAs (Jedrzejczyk et al., 2017).

The RNAi mechanism is a natural process and the initial molecule is mostly hairpin and matched parts in the intron regions of the primary mRNA are synthesized by double-chain RNA or RNA polymerase II. RNA-bound RNA polymerase synthesizes these using endogenous or exogenous-derived RNA (Bartel, 2004). With the discovery that siRNAs were made from exogenous dsRNA through the RNAi mechanism, it is realized that miRNAs and endogenous siRNAs regulate gene expression. SiRNAs, 21-23 nt long, are endogenous and exogenous and serve as guide RNA for proper mRNA degradation (Tomari and Zamore, 2018, Doench et al., 2018). Although chemically similar to miRNAs, siRNAs are generated by dicer by dividing long, double-strand RNAs. siRNAs are 2 nt shorter at the 5' phosphate end and 2 nt long at the 3' hydroxyl end. siRNA and RISC complex hybridize with target mRNA according to the principle of Watson-Crick base mapping. The RISC complex contains complementary sequences to 10 nt at 5' ends of single-strand siRNA. Endonucleases in the RISC complex degrade mRNA. In other words, siRNAs function in RNAi within an RNA-bound silencer complex (RISC) (Carrington et al., 2003).

RNA initiates the first step of interference and the Dicer, which belongs to the RNAse III ribonuclease enzyme family, allows double-strand RNA to be cleaved into small silencing (siRNA) RNAs (Song and Rossi, 2017). These RNAs are then transferred to the RISC complex in an ATP dependent manner. RISC is an RNA-multiprotein complex with nuclease activity, binds to siRNAs, and is directed to mRNA accompanied by the appropriate siRNA chain (Doench et al., 2018). The interaction of siRNA or miRNA with mRNA also occurs within the RISC complex. siRNA

matches the target mRNA sequence one-on-one, and the mRNA molecule is cleaved from the matching regions with endonucleases and removed. If the substrate is miRNA, the target can be matched (i.e., partially) with certain nucleotides in the 3'-non-translational region of mRNA. The most critical match is seen in the approximately 8 nt at the 5' end of miRNA. When paired with the appropriate mRNA chain, mRNA degradation occurs, thereby silencing the gene. In addition to being a natural process in the living organism, RNA interference suppresses the expression of endogenic genes by using siRNAs in *in vitro* conditions and this suppression is important in the research of gene functions. It provides genome identification in species and help determine the presence of many unknown genes. The RNAi mechanism is an ideal technique in genome research. Suppression of gene expression is very important in researching gene function and gene therapy (Zamore P, 2000; Raja et al., 2019; Xu et al., 2019).

miRNA Uses

miRNAs are associated with post-transcriptional regulation of gene transcription, differentiation, control of growth, apoptosis, and oncogenes. It was found to play a role tumorigenesis and in the development of the organisms (Melo and Melo, 2013). It is also reported to play a role in a wide range of developmental processes, including cell-division, cell proliferation, cell differentiation, apoptosis, development, and neuronal regeneration and differentiation. In addition, it is reported that upregulated miRNA expression results in oncogenesis as well as downregulated expression may cause tumor suppression, thereby regulating various characteristics of cancer such as angiogenesis, apoptosis, cell proliferation, differentiation, etc. miRNAs are involved in regulating heart function and in the functioning of the cardiovascular system of mammals. It has been found that the miRNAs have important roles in every step of development of the central nervous system (CNS) neurogenesis and are important in brain development, as well as the pathogenesis of neuronal diseases (Dwivedi et al., 2019). In humans, miRNAs were found to exist in all chromosomes except the Y chromosome (Melo and Melo, 2013). In general, some miRNAs have an obvious role in vertebrate development, while some miRNAs are effective in physiological and cellular processes (Wienholds and Plasterk, 2005).

Conclusion

The importance of miRNAs in proliferation and cell differentiation, as well as abnormal conditions such as cardiovascular diseases, diabetes, and cancer is indicated. Recently, there have been great advances in the number of studies and the use of more precise techniques to determine the relationship of miRNAs with targeted genes and diseases. Further understanding of the molecular pathways of miRNA is thought to be necessary in advancing therapeutic treatments for other diseases, especially cancer. Together with all this information, the importance of miRNAs has become indisputable.

Acknowledgements

We would like to thank Architect Büşra SUVARIKLI SOLAK for her help in the drawing the figure in this review.

Conflict of Interest

The authors declare that they have no conflict of interests.

References

Agrawal, N., Dasaradhi, P.V., Mohmmed, A., Malhotra, P., Bhatnagar, R.K., & Mukherjee, S.K. (2003). RNA interference: biology, mechanism, and applications. *Microbiology and Molecular Biology Reviews,* 67 (4), 657-85. doi: 10.1128/MMBR.67.4.657-685.2003

Alvarez-Garcia, I., & Miska, E.A. (2005). MicroRNA functions in animal development and human disease. *Development,* 132 (21), 4653-62. doi: 10.1242/dev.02073

Ambros, V., Bartel, B., Bartel, D.P., Burge, C.B., Carrington, J.C., Chen, X., Dreyfuss, G., Eddy, S.R., Griffiths-Jones, S., Marshall, M., Matzke, M., Ruvkun, G., & Tuschl, T. (2003). A uniform system for microRNA annotation. *RNA*, 9 (3), 277-9. doi: 10.1261/rna.2183803

Bartel, D.P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116 (2), 281-97. doi: 10.1016/s0092-8674(04)00045-5

Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory functions. *Cell*, 136 (2), 215-33. doi: 10.1016/j.cell.2009.01.002

Bernstein, E., Caudy, A.A., Hammond, S.M., & Hannon, G.H. (2001). Role For A Bidentate Ribonuclease in The Initation Step of RNA Interference. *Nature*, 409, 363- 366. doi: 10.1038/35053110

Betancur, J.G., & Tomari, Y. (2012). Dicer is dispensable for asymmetric RISC loading in mammals. *RNA*, 18 (1), 24-30. doi: 10.1261/rna.029785.111

Betel, D., Wilson, M., Gabow, A., Marks, D.S., & Sander, C. (2007). The microRNA.org resource: targets and expression. *Nucleic Acids Research*, 36, 149-153. doi: 10.1093/nar/gkm995

Cai, X., Hagedorn, C.H., & Cullen, B.R. (2004). Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA*, 10 (12), 1957-66. doi: 10.1261/rna.7135204

Carrington, J.C., & Ambros, V. (2003). Role of microRNAs in plant and animal development. *Science*, 301**,** 336–338. doi: 10.1126/science.1085242

Carthew, R.W., & Sontheimer, E.J. (2009). Origins and Mechanisms of miRNAs and siRNAs. *Cell*, 136 (4), 642-55. doi: 10.1016/j.cell.2009.01.035

Collins, R.E., & Cheng, X. (2005). Structural domains in RNAi. *FEBS Letters*, 579 (26), 5841-9. doi.org/10.1016/j.febslet.2005.07.072

Court, D.L., Gan, J., Liang, Y.H., Shaw, G.X., Tropea, J.E., Costantino, N., Waugh, D.S., & Ji, X. (2013). RNase III: Genetics and function; structure and mechanism. *Annual Review of Genetics,* 47, 405-31. doi: 10.1146/annurev-genet-110711-155618

Cowland, J.B., Hother, C., & Gronbaek, K. (2007). MicroRNAs and cancer. *APMIS*, 115 (10), 1090-106. doi: 10.1111/j.1600-0463.2007.apm_775.xml.x

Denli, A.M., & Hannon, G.J. (2003). RNAi: an ever-growing puzzle. *Trends in Biochemical Sciences,* 28 (4), 196-201. doi: 10.1016/S0968-0004(03)00058-6

Doench, J.G., Petersen, C.P., & Sharp, P.A. (2018). siRNAs can function as miRNAs. *Genes & Development*, 17 (4), 438–42. doi: 10.1101/gad.1064703

Du, T., & Zamore, P.D. (2005). microPrimer: the biogenesis and function of microRNA. *Development*, 132 (21), 4645-52. doi.org/10.1242/dev.02070

Dwivedi, S., Purohit, P., & Sharma, P. (2019). MicroRNAs and Diseases: Promising Biomarkers for Diagnosis and Therapeutics. *Indian Journal of Clinical Biochemistry*, 34 (3), 243-5. doi: 10.1007/s12291-019-00844-x

Ghildiyal, M., & Zamore, P.D. (2009). Small silencing RNAs: an expanding universe. *Nature Reviews Genetics,* 10 (2), 94-108. doi: 10.1038/nrg2504

Graves, P., & Zeng, Y. (2012). Biogenesis of Mammalian MicroRNAs: A Global View. *Genomics Proteomics Bioinformatics.* 10 (5), 239–245. doi: 10.1016/j.gpb.2012.06.004

Fire, A., Xu, S.Q., Montgomery, M.K., Kostas, S.A., Driver, S.E., & Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature,* 391, 806-811. doi: 10.1038/35888

Ha, M., & Kim, V.N. (2014). Regulation of microRNA biogenesis. *Nature Reviews Molecular Cell Biology,* 15 (8), 509-524. doi:10.1038/nrm3838

Hall, T.M. (2005). Structure and function of argonaute proteins. *Structure,* 13 (10), 1403-1408. doi: 10.1016/j.str.2005.08.005

Han, J., Lee, Y., Yeom, K.H., Kim, Y.K., Jin, H., & Kim, V.N. (2004). The Drosha-DGCR8 complex in primary microRNA processing. *Genes & Development,* 18 (24), 3016-3027. doi: 10.1101/gad.1262504

Höck, J., & Meister, G. (2008). Protein family review The Argonaute protein family. *Genome Biology,* 9 (2),210, doi: 10.1186/gb-2008-9-2-210

Hutvagner, G., & Simard, M.J. (2008). Argonaute proteins: key players in RNA silencing. *Nature Reviews Molecular Cell Biology*, 9 (1), 22-32. doi: 10.1038/nrm2321

Hydbring, P., & Badalian-Very, G. (2013). Clinical applications of microRNAs. *F1000 Research*, 6 (2), 136. doi: 10.12688/f1000research.2-136.v3

Jansson, M.D., & Lund, A.H. (2012). MicroRNA and cancer. *Molecular Oncology,* 6 (6), 590-610. doi.org/10.1016/j.molonc.2012.09.006

Jedrzejczyk, D., Gendaszewska-Darmach, E., Pawlowska, R., & Chworos, A. (2017). Designing synthetic RNA for delivery by nanoparticles. *Journal of Physics: Condensed Matter,* 29 (12), 123001. doi: 10.1088/1361-648X/aa5561

Karginov, F.V., Cheloufi, S., Chong, M.M., Stark, A., Smith, A.D., & Hannon, G.J. (2010). Diverse endonucleolytic cleavage sites in the mammalian transcriptome depend upon microRNAs, Drosha, and additional nucleases. *Molecular Cell*, 38 (6), 781-788. doi: 10.1016/j.molcel.2010.06.001

Kim, D., & Rossi, J. (2008). RNAi mechanisms and applications. *Biotechniques,* 44 (5), 613-616. doi: 10.2144/000112792

Kim, V.N., Han, J., & Siomi, M.C. (2009). Biogenesis of small RNAs in animals. *Nature Reviews Molecular Cell Biology*, 10 (2), 126-139. doi: 10.1038/nrm2632

Krol, J., Loedige, I., & Filipowicz, W. (2010). The widespread regulation of microRNA biogenesis, function and decay. *Nature Reviews Genetics,* 11 (9), 597-610. doi: 10.1038/nrg2843

Kwon, S.C., Nguyen, T.A., Choi, Y.G., Jo, M.H., Hohng, S., Kim, V.N., & Woo, J.S. (2016). Structure of Human DROSHA. *Cell,* 164 (1-2), 81-90. doi: 10.1016/j.cell.2015.12.019

Lee, R.C., Feinbaum, R.L., & Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell,* 75 (5), 843–854. doi: 10.1016/0092-8674(93)90529-y

Lei, E.P., & Silver, P.A. (2002). Protein and RNA export from the nucleus. *Developmental Cell*, 2 (3), 261-272. doi.org/10.1016/S1534-5807(02)00134-X

Lucas, K., & Raikhel, A.S. (2013). Insect microRNAs: biogenesis, expression profiling and biological functions. *Insect Biochemistry and Molecular Biology*, 43 (1), 24-38. doi: 10.1016/j.ibmb.2012.10.009

MacFarlane, L., & Murphy, P.R. (2010). MicroRNA: Biogenesis, Function and Role in Cancer. *Current Genomics,* 11 (7), 537-561. doi: 10.2174/138920210793175895

MacRae, I.J., Zhou, K., Li, F., Repic, A., Brooks, A.N., Cande, W.Z., Adams, P.D., & Doudna, J.A. (2006). Structural basis for double-stranded RNA processing by Dicer. *Science,* 311 (5758), 195-198. doi: 10.1126/science.1121638

Macrae, I.J., Li, F., Zhou, K., Cande, W.Z., & Doudna, J.A. (2006). Structure of Dicer and mechanistic implications for RNAi. *Cold Spring Harbor Symposia on Quantitative Biology,* 71 (71), 73-80. doi: 10.1101/sqb.2006.71.042

MacRae, I.J., & Doudna, J.A. (2007). Ribonuclease revisited: structural insights into ribonuclease III family enzymes. *Current Opinion in Structural Biology*, 17 (1), 138-145. doi: 10.1016/j.sbi.2006.12.002

Mattick, J.S., & Makunin, I.V. (2006). Non-coding RNA. *Human Molecular Genetics,* 15 (1), 17-29. doi.org/10.1093/hmg/ddl046

Melo, C.A., & Melo, S.A. (2013). Biogenesis and Physiology of MicroRNAs. *Non-coding RNAs and Cancer*, 5-24. doi: 10.1007/978-1-4614-8444-8_2

Mortimer, S.A., Kidwell, M.A., & Doudna, J.A. (2014). Insights into RNA structure and function from genome-wide studies. *Nature Reviews Genetics,* 15 (7), 469-79. doi: 10.1038/nrg3681

Napoli, C., Lemieux, C., & Jorgensen, R. (1990). Introduction of a chimeric chalcone synthase gene into Petunia result in supression of homologous revesible co-supression of homologous genes in trans. *The Plant Cell,* 2 (4), 279-289. doi: 10.1105/tpc.2.4.279

Olena, A.F., & Patton, J.G. (2010). Genomic organization of microRNAs. *Journal of Cellular Physiology,* 222 (3), 540-545. doi: 10.1002/jcp.21993

Pillai, R.S. (2005). MicroRNA function: multiple mechanisms for a tiny RNA?, *RNA*, 11 (12), 1753-1761. doi: 10.1261/rna.2248605

Raja, M.A.G., Katas, H., & Amjad, M.W. (2019). Design, mechanism, delivery and therapeutics of canonical and Dicer-substrate siRNA. *Asian Journal of Pharmaceutics Science,* 14 (5), 497-510. doi.org/10.1016/j.ajps.2018.12.005

Santosh, B., Varshney, A., & Yadava, P.K. (2014). Non-coding RNAs: biological functions and applications. *Cell Biochemistry & Function,* 33 (1), 14-22. doi: 10.1002/cbf.3079

Shabalina, S.A., & Koonin, E.V. (2008). Origins and evolution of eukaryotic RNA interference. *Trends Ecology Evolution,* 23 (10), 578-587. doi: 10.1016/j.tree.2008.06.005

Shukla, G.C., Singh, J., & Barik, S. (2011). MicroRNAs: Processing, Maturation, Target Recognition and Regulatory Functions. *Molecular And Cellular Pharmacology,* 3 (3), 83-92. doi: 10.4255/mcpharmacol.11.13

Singh, S.K., Pal Bhadra, M., Girschick, H.J., & Bhadra, U. (2008). MicroRNAs--micro in size but macro in function. *The FEBS Journal,* 275 (20), 4929-4944. doi: 10.1111/j.1742-4658.2008.06624.x

Song, M.S., & Rossi, J.J. (2017). Molecular mechanisms of Dicer: endonuclease and enzymatic activity. *Biochemical Journal,* 474 (10), 1603-18. doi: 10.1042/BCJ20160759

Tetreault, N., & De Guire, V. (2013). miRNAs: their discovery, biogenesis and mechanism of action. *Clinical Biochemistry*, 46 (10-11), 842-845. doi: 10.1016/j.clinbiochem.2013.02.009

Tijsterman, M., & Plasterk, P.H. (2004). Dicers at RISC; the mechanism of RNAi, *Cell,* 117 (1), 1-3. doi: 10.1016/s0092-8674(04)00293-4

Tomari, Y., & Zamore, P.D. (2018). Perspective: machines for RNAi. *Genes & Development,* 19 (5), 517–529. doi: 10.1101/gad.1284105

Vermeulen, A., Behlen, L., Reynolds, A., Wolfson, A., Marshall, W.S., Karpilow, J., & Khvorova, A. (2005). The contributions of dsRNA structure to Dicer specificity and efficiency. *RNA*, 11 (5), 674-682. doi: 10.1261/rna.7272305

Wahid, F., Shehzad, A., Khan, T., & Kim, Y.Y. (2010). MicroRNAs: synthesis, mechanism, function, and recent clinical trials. *Biochimica et Biophysica Acta*, 1803 (11), 1231-43. doi: 10.1016/j.bbamcr.2010.06.013

Wienholds, E., & Plasterk, R.H. (2005). MicroRNA function in animal development. *FEBS Letters*, 579 (26), 5911-5922. doi: 10.1016/j.febslet.2005.07.070

Williams, T., Ngo, L.H., & Wickramasinghe, V.O. (2018). Nuclear export of RNA: Different sizes, shapes and functions. *Seminars in Cell and Developmental Biology,* 75, 70-77. doi: 10.1016/j.semcdb.2017.08.054

Wilson, R.C., & Doudna, J.A. (2013). Molecular mechanisms of RNA interference. *Annual Review of Biophysics,* 42 (1), 217-239. doi: 10.1146/annurev-biophys-083012-130404

Wightman, B., & Ha, I.G.R. (1993). Posttranscriptional Regulation of the Heterochronic Gene lin-14 by W-4 Mediates Temporal Pattern Formation in C. elegans. *Cell*, 75 (5), 855-862. doi: 10.1016/0092-8674(93)90530-4

Xu, W., Jiang, X., & Huang, L. (2019). RNA Interference Technology. In:

Comprehensive Biotechnology, 560-575. doi: 10.1016/B978-0-444-64046-8.00282-2

Zamore, P.D., Tuschl, T., Sharp, P.A, & Bartel, D.P. (2000). RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell,* 101 (1), 25-33. doi: 10.1016/S0092-8674(00)80620-0