
 Review Article

New Advances in RNAs

Moslem Bahadori MD*

Since the discovery of the first microRNA gene, *lin-4*, in *Caenorhabditis elegans* in 1993, many of this small regulatory genes have been reported in plants, viruses, and various kinds of animals. This announced a new world of RNA molecules, which subverted our traditional thinking about RNA. In this review, I summarized the main findings from researchers at different laboratories on microRNAs importance, functionality, production, and different aspects of their biologic activities.

A narrative literature overview of relevant papers known to the author and that were retrieved from PubMed is presented.

MicroRNA, a nonprotein-coding small RNA with almost 21 – 23 nucleotides in length, is an essential regulatory apparatus in the cells and their environment. They are crucial molecules for development, evolution, cellular differentiation, proliferation, embryogenesis, and cell death. Two classes of microRNAs exist: small temporal RNA—stRNA (miRNAs) and small interfering RNA—siRNAs (RNAi). The latter is a useful tool for the diagnosis, treatment, and prevention of diseases particularly cancer and viral diseases.

There have been tremendous research regarding various aspects of these genes silencing and regulatory molecules which are preserved in all kinds of creatures. Currently, about 1% of the known human genes encode microRNAs.

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Introduction

In the opening session of the Annual Congress of Iranian Pathologists, there is usually a brief report of the emerging technologies or scientific innovations on the field of medicine and/or pathology. At the 9th Annual Congress held in November 7 – 9, 2007 in Tehran, the subject was “New Advances in RNAs.”¹ The following is an overview of that report.

Traditionally, biologists believed that ribonucleic acid (RNA) molecules, as mRNA, simply carried genetic information from DNA in the nucleus to the places in the cell where proteins are made. The simple formula was believed to be: DNA makes RNA, RNA produces protein, and proteins are major cellular machinery that carry out all the crucial tasks, as structural substances, enzymes, hormones, and so on. RNAs were also

founded in the protein factory themselves. Two other molecules of the RNAs family are nonprotein coding and are called rRNA and tRNA. With this idea, the major efforts of gene hunter researchers were to discover new structural genes whose products encode proteins.²⁻⁴

When the human-genome project was completed, biologists wondered to see that plants and animals, of any species, all seem to have approximately the same amount of genes to produce proteins. Yet, the complexities of all are very different. Flies are more complex than worms and they are much less complex than mammals. In addition to the above, the recent studies indicated that nearly 97% of the human genome is composed of noncoding DNA, which varies from one species to another. Instead, many of them produce important noncoding regulatory products. The most recent among this nonprotein-coding RNA (npcRNA) class are genes that encode small or short- length RNA molecules (approximately <40 nucleotide in length). An important group of these are called microRNAs. These molecules unlike mRNAs, do not encode protein but inhibit mRNA

Authors' affiliation: *Academy of Medical Sciences of I.R. of Iran, Tehran, Iran.

Corresponding author and reprints: Moslem Bahadori MD, Academy of Medical Sciences of I.R. of Iran, Tehran, Iran. Tel: +98-212-293-8051, E-mail: bahadori@ams.com

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gene production.⁵⁻¹⁰

History

Short-length RNAs (small RNAs) include many different classes of nonprotein-coding RNA molecules. Among these are microRNAs (miRNAs) and small interfering RNAs (siRNAs), each with their own properties and functions. These molecules function in many physiologic and pathologic processes. The main function of this class of RNA is regulatory function in the form of interfering with protein production of target mRNAs either via specific cleavages of homologous mRNA or by specific inhibition of mRNA protein synthesis.⁵

The research which led to discovery of these molecules started in 1993.^{4,8} Investigators were working with a nematode called *Caenorhabditis elegans*; they were looking how the developmental timing of this worm is controlled. They found a small temporal RNA (stRNA) that was concerned with this process, and labeled this as lin-4.¹¹⁻¹⁴ They observed that stRNAs do not encode protein but their actual function was not clear. Later on, they found that the molecule was involved with down-regulation of mRNA that specified the temporal progression during development. Wightman et al. identified the first microRNA target lin-14 mRNA.⁸ Another stRNA called let-7 was discovered in 2000 which has had the similar effects.¹⁴ Let-7 was expressed during the fourth stage of larval *C. elegans* development. Still the importance of small RNAs was not known. Attentions grew when they found that homologous of let-7 was present in other species such as frogs, flies, mice, and human, and the sequences of all this let-7 RNA was similar and in many cases identical. Lin-4 and let-7 were identified by their mutant phenotype and until recently they were the only known small RNA.¹⁵ Flow of investigations in various research laboratories in the world led to more understanding and precise definition of lin-4, let-7, and short-length RNAs. The finding in this regards showed that nonprotein-coding small RNAs have mainly a gene-silencing property that is preserved in all livings, and the genes have thus been called "silencing genes." Shortly after the discovery of the first short-length RNA gene, lin-4, in *C. elegans*, many such regulatory RNA genes have been identified in worms, plants, flies, fish, mammals, and human. Andrew Fire and Craig Mello, among other investigators, have been

awarded the 2006 Noble Laureate for Physiology and Medicine for their discovery.¹⁶⁻¹⁷

The recent identification of small short-length RNA, which is discovered in nearly all creatures, has revolutionized our understanding of gene performance, activities, and regulation. Small RNA interference (siRNAs) and related RNA silencing phenomenon with experimental introduction of RNAi into cells to interfere with function of an endogenous gene have provided great hope for understanding the physiology and pathology of cells.¹⁸⁻²¹

Definition

MicroRNAs are single-stranded small RNA molecules 22 – 23 nucleotides (nt) in length.^{4,22-24} These are noncoding RNAs that are negatively regulating gene expression via sequence-specific base pairing with target mRNAs. miRNAs are of RNA family, encoded by genes that are transcribed from DNA (see below) but not translated into protein.

siRNAs are another small RNAs and mediate the phenomenon of RNA interference called RNAi. It was first discovered in plants and lower organisms as an indigenous evolutionary conserved biologic molecule.²²⁻²⁴ According to Lagos-Quintana et al. siRNAs are double-stranded but stRNA are single-stranded.²⁰ Small RNAs have diverse expression pattern during development.

miRNAs and siRNAs are created in the same pattern. Both stRNAs and siRNAs are generated by a process requiring Dicer, a pronuclease III enzyme. Dicer is a multidomain protein with tandem ribonuclease III (RNase III) domain. Dicer processes double-stranded RNA into small fragments about 21 – 25 nucleotides (nt) in length. Dicer is composed of three structurally rigid regions connected by flexible hinge.²⁵ miRNAs and siRNAs mediate down-regulation of mRNA gene expression. But the way they act in mRNAs are different. Since miRNAs have evolutionarily preserved regulatory functions, they are featuring orthologs in other species.⁵ miRNA biogenesis and function specificity and potency rely on the nature of protein-protein, protein-RNA, and RNA-RNA interactions found in different complexes. Any of these will fulfill a specific function in a well-orchestrated process.²⁶

Production of small RNAs

Two distinct pathways exist for the action of

short-length RNAs (stRNA and siRNAs), but their production is more or less similar. Here, we briefly consider the production of miRNAs.^{4,27}

MicroRNAs are produced through transcription of miRNA genes in the nucleus known as microRNAs precursor genes (mir-gene). Rigoutsos and his group of IBM Research Center Studies predicted that the number of microRNA precursors in mammalian genomes likely ranges in the tens of thousands.^{25, 28, 29} The genes encoding microRNAs are much longer than the processed mature miRNA molecules.

1- Transcription of miRNA genes (mir-gene) produces primary miRNA transcript (pri-miRNA). Pri-miRNA has a cap and poly-A tail.

2- Primary miRNAs are processed in nucleus to short, 70-nt loop structure, the so-called "pre-miRNAs." This process is performed by a protein complex named microprocessor complex consisting of the nuclease Drosha and stranded RNA-binding protein Pasha³⁰ or as it is performed in mammals, this can be done through pronuclease III, a Dicer. The microprocessor complex varies according to creature, for example, the pathway in plants varies slightly due to their lack of Drosha homolog.

3- With the help of specific transporter proteins (i.e., exportin-5 complex) pre-miRNA is exported to the cytoplasm.

4- Pre-miRNAs through an additional "cutting" by an enzyme Dicer generate mature miRNAs. Dicer also initiates the formation of the RNA-induced silencing complex (RISC).^{30,31} Mature miRNAs are about 21–30 nt in length. At this stage, miRNAs are still double stranded.

5- With the action of Dicer miRNAs unwind and make two single-stranded molecules.

6- Single-stranded molecules of this duplex are incorporated into a multiprotein complex known as RISC.

7- Base pairing between single-stranded miRNAs/RNAi and target mRNA direct RISC to either.

- a- repress target mRNA translation, or
- b- mRNA destruction (cleavage).

In either ways, the gene from which the target mRNA was derived is silenced. A given miRNA can silence many target mRNA genes, estimate range from one to hundred target genes, based on target predictions using a variety of bioinformatics approaches.^{32,29} For example, thousands of mammalian mRNAs are under selective pressure

maintaining 7-nt sites matching miRNAs. As a consequence a single miRNA, direct or indirect, regulates many of these target sites.³³

Silencing performance

MicroRNAs are responsible for post-transcriptional gene silencing as part of critical cellular pathways and intercellular coordination, mainly during embryonic development.^{34–36}

Silencing of gene expression by microRNAs is preserved in all living creatures, from flowering plants³⁷ to human. The basic mechanism of silencing phenomenon is still not known. It is not understood to what extent and through what process does the suppression of mRNA or reduction of its activity occur. Gene silencing is a fundamental mechanism of gene regulation, mastering creation development. In mammals, the majority of miRNAs guide RISC to the 3' untranslated regions (UTRs) of mRNA targets, with the consequence that translation of the target mRNA is inhibited.³⁸

miRNAs and siRNAs involving in RNAi use the same RISC to direct silencing. For miRNAs processing, its precursor double-stranded RNA (dsRNA), uses Dicer enzyme which will produce single-stranded small RNA. This small RNA attaches to RISC and directs to target mRNA. After that the mechanism diverges, miRNAs attach imperfectly to mRNA and form a bulge that block mRNA to produce protein while siRNA binds perfectly with the target mRNA and destroy mRNA.⁴

The cells are full of RNAs

The number and the diversity of RNAs are amazingly numerous and many more miRNAs and siRNAs are being reported.

Tuschl group of Max Plank Institute identified 16 novel miRNAs which are only expressed in 0 – 2-hr embryo of *Drosophila melanogaster*.²⁰ Each clusters and family of short-length small RNAs have many subsets. New RNAs are crushing forth from research laboratories rapidly. The subset of microRNAs have their own specific function. Examples are:

- siRNAs-like scan RNA or scanRNAs (scnRNAs) are produced from nongenic, heterogeneous micronuclear transcripts, take part in genome rearrangements, chromosome segregation, and meiotic prophase.^{39,40}
- Small nuclear RNAs (snRNAs) are constituent

of spliceosome (a large, dynamic ribonuclear protein complex, which contains five essential snRNAs from U1 to U7), the mechanism involved to produce mRNA by removing introns region of genes.⁴¹⁻⁴³

- Small nucleolar RNAs (snoRNAs) have been identified in rice and other plants. These are involved in rRNA processing. It modifies ribosomal RNAs by orchestrating the cleavage of the long pre-rRNA into the functional subunits.⁴⁴⁻⁴⁶
- Repeat-associated siRNAs (rasiRNAs) silence the genetic repeat. It has been suggested that rasiRNAs-based silencing is mechanistically different miRNAs and RNAi pathway.⁴⁷⁻⁴⁹
- Transacting short interference RNAs (tasiRNA). They have been distinguished recently. There are genes encoding tasiRNA cleavage plant developing proteins. TasiRNAs regulate gene expression at the post-transcriptional level.^{49,50}
- Natural antisense transcript-associated siRNAs (nat siRNAs). These were discovered in plant and there are human homologous for it.^{49,50}
- piwi-interacting RNAs (piRNAs) (which were introduced last summer), are abundant in developing sex cells. piRNAs have been identified as key players in germline. No male mammal, male fish or fly of either sex would be fertile without them. Mammalian piRNAs are approximately 26 – 31 nt in length.⁵¹⁻⁵³
- X-inactive specific transcript RNAs (xistRNAs) have important roles in X-chromosome inactivation (XCI) and have the power to turn off an entire chromosome. In female, the action is more severe because of two X-chromosomes.⁵⁴⁻⁵⁷
- Pregnancy-induced noncoding RNAs (pincRNAs). These newly-found miRNAs have been seen in pregnancy.

Hundreds of papers during the last three to four years have been published regarding various activities of these new molecules. Small noncoding RNAs of the miRNAs class appear to be numerous and diverse.¹⁵ According to Isidro Rigoutsos of IBM, more than 37,000 different microRNAs present compares with 21,000 or so protein-encoding genes.³ This is not an exaggeration when we consider that 97% of human genome is composed of noncoding DNA. Furthermore, the results of a project called ENCODE published in Nature (searching the detail of only 1% of human

genome) nearly accepted the amount of genes dealing with protein coding may be less than that was estimated.

MicroRNAs and embryonic stem cells (ESC)

Stem cells have the capability to divide throughout their life to produce differentiated daughter cells while maintaining a population which are undifferentiated and remain stem cell in its proper niche. Stem cells possess the ability to produce any types of cells. They are divided as embryonic stem cells (ESCs), adult tissue stem cells, and many cancers also have small population of cells with the character of stem cells. These cells have been called cancer stem cells and thought to be the cause of cancer relapse.⁵⁸ MicroRNAs are considered crucial and thought to be the key to understanding how stem cells remain in a state from which they can produce any types of cells. miRNAs have also been implicated in the development and pathology of stem cells.⁵⁸ Multipotential mesenchymal stromal cells (MSCs) ,which can be isolated from adult tissue, have the capability for self-renew and differentiate into different lineages. Both of them are under regulation of miRNAs.⁵⁹

Dr. Haifan Lin, one of the chief investigators in cell biology at Yale University, studied the mechanism of stem cell self-renewal in fruits, flies, mice, and human cancer cells. He discovered that a group of piRNAs may play an important role in stem cell proliferation and germline development. Around 20 miRNAs are made only in human ESC. The miRNA expressions vary in both differentiated and undifferentiated stem cells.⁵⁸⁻⁶¹

RNA interference (RNAi)

Development of gene-specific double-stranded RNAs (dsRNAs) is the base of these newly-recognized silencing RNAs. These are a unique class with relative stability, that have the ability to activate the interferon pathway and in particular production of RNA interference (RNAi).⁶²⁻⁶³ RNAi, was discovered in 1998⁶⁴; it is a preserved ancient silencing gene with diverse actions and activities.

RNAi's action is to turn down the production of any single protein to very low levels and that distinguishes it from miRNAs which control multiple proteins simultaneously. RNAi mechanistically knocks down gene expression via different actions including mRNA degradation,

inhibition of its translation, or chromatin remodeling. It seems that these could be powerful tools for generating deficient phenotypes without mutating genes. In this way it becomes promising hope for future treatment of the diseases. This will provide a roadmap launches RNAi from research bench to the bedside medicine^{24,62-64} and drugs based on RNAi are moving fast toward pharmaceutical market (see below).

Functional features of miRNAs

In plants and animals, microRNAs are essential post-transcriptional gene regulators of various processes such as proliferation, differentiation, development, programmed cell death, and interaction between virus and host cells.⁶⁴ miRNAs regulate the expression of downstream gene targets including transcriptional factors. miRNAs regulate the activity of at least one third of human protein-encoding genes. But there is very little cellular proteins that do not happen under direct microRNA watch, since other microRNAs derivatives such as siRNAs do.

MicroRNAs are also regulated by extracellular signaling pathways that are important for differentiation into specific tissue, suggesting that they play a role in specifying tissue identity. Expression of a known miRNA in a specific cell type plays a useful marker for identifying a particular cell type.⁵⁹

The complexities of creature

As is known, the current concept is that DNAs store data that will get translated into living organisms. In this manner, the complexity of the development related to genes is presented in DNAs: more genes give more complexities.

Now, it become clear that all living creatures have about the same number of genes with little differences to produce proteins; nevertheless, their complexities vary. *C. elegans*, a tiny worm that lacks a proper brain or a *Drosophila*, a fruit fly, have nearly the same number of genes that human has—only a little bit shorter. Thus, it seems that the traditional gene propensities are not as only important molecules as it was believed. Instead, there must be other molecules involved in this connection that is the regulatory genes, which are related to RNA operating system. The researchers discovered that it is not the function of protein-encoding RNAs (mRNAs) *per se*, but it is the regulatory RNA system which is grouped as short-

length RNAs or microRNAs family. In regard of complexities, it does not matter how many protein-coding genes you have; it is important how they are regulated. By silencing target protein-coding RNAs, microRNAs explain why some creatures are more complex than others. What exactly those microRNAs molecules do, have been the subjects of tremendous investigations. Single-stranded miRNAs regulate the levels and qualities of hundred different proteins. As has been mentioned earlier, they are like “powerful strings controlling copious protein puppets”.³ Some types of these regulatory miRNAs (or siRNAs) edit other kinds of microRNAs. So far, approximately 500 known mammalian miRNAs gene families have been identified; each of these gene families may regulate hundreds of different protein-coding genes. According to a guess estimate, there are approximately 1,000 genes out of 30,000 human genes that encode small RNAs and up to 30% of human protein-coding genes may be regulated by microRNAs.

MicroRNAs and our understanding of diseases

An interesting consequence of recognition of small RNAs is that researchers found a new source for understanding disease processes.³² Accordingly, they have found aberrant or altered miRNAs expressions involving a number of human diseases including malignant, viral, infectious, metabolic, and respiratory and heart diseases.⁶⁵⁻⁶⁸ Many miRNAs are deregulated in human tumors⁶⁷; many tumor miRNAs are located in the genomic regions linked to cancer.^{53,68} The role of miRNAs in cancer includes oncogenes and tumor suppressor genes. Identifying proper biomarkers makes them appealing targets for diagnosis, therapy, and prediction of the outcome. miRNAs signature can be used to detect and classify various neoplastic tumors and with certain profiles of miRNA expression one can predict the outcome of a disease. It become a new promise for anticancer gene therapy.^{38,52} The discovery of miRNAs encoded by a family of virus oncogene, and the finding that numerous DNA viruses also encode miRNAs, attracted scientists' attention to the probability that viruses themselves have their own miRNAs and miRNAs as critical mediator for viral action including oncogenicity.^{69,70} In another view, researchers showed that viral gene expression is down-regulated by host miRNA which could be a promise for design and manufacturing new

antiviral drugs.

RNAs in the world of treatment

The new RNAs world is also a source for the treatment of diseases. In this approach, the basic technology is RNAi technology that is the sequence-specific gene silencing. RNAi is made of small interfering RNAs (siRNAs) which are derived from longer dsRNA by a Dicer. RNAi can be applied as man-made or be used as endogenous molecules. RNAi becomes widely applied as experimental tools to analyze, both *in vitro* and *in vivo*, the function of genes. RNAi has been produced synthetically in mammalian cells by introduction of manufactured either double-stranded siRNAs or by plasmid and viral vector systems that express short hairpin double-stranded RNAs (shRNA) which are subsequently by cell machinery processed to siRNAs.²⁴ Experimentally, in mammalian cells, RNAi has been used to clarify the functional role of individual genes. This has been done in different diseases particularly in cancer cells and brain degenerative diseases. As is known, cancer involves mutant genes that cause uncontrolled cell proliferation. The aim for researchers is to silence those genes with RNAi. Most of these trials have been done on cell culture in the laboratory.⁷¹ The main objectives are to turn from laboratory to bedside. Apart from direct therapeutic action of RNAi, some RNAi therapies may be used to support conventional chemotherapy. Whitehurst and his group at the University of Texas used RNAi for this purpose. They converted tumoral cells that did not respond to anticancer drug paclitaxel into cells that become sensitive to this drug⁷¹ and this could be promising for other drugs or multidrug-resistant diseases.

RNAi drugs stop the production of disease-related protein at source. In this regard, they are now being tested as new tools in many pharmaceutical companies searching for new drugs, though it is currently in its introductory phase. Many companies including Opko Corporation, Novartis, and Glaxo Smith Kline, are planning to gain new markets in this field. Some of them doing final round of their clinical trials for an RNAi-based drug.³ Novartis already announced its successes on several diseases such as macular degeneration of eye, hepatitis C, Huntington's disease, respiratory infection, and cancer.⁷²

The most probable explanation for RNAi is that it acts as a defense against viruses including HIV.

RNAi is a vital component interacting with the innate anti-inflammatory and antiviral immune response. In human interferon beta (β -IFN) has been shown to be involved with the expression of many cellular miRNAs and they have been sequenced in HCV genomic RNA.⁷³⁻⁷⁴

RNAs, unlike DNAs, are single-stranded and double-stranded RNA (dsRNA) is rare in nature. However, viruses often make it when they reproduce. It appears that organisms with the ability to recognize and destroy dsRNA molecules (such as virus) will destroy dsRNAs; RNAi has this ability. Using siRNA in cell cultures, Bitko and his group in an experiment induced prevention and inhibition of respiratory viruses (RSV and parainfluenza, PIV) by nasally-administered siRNAs.⁶⁸

Small RNAs and evolution

Alvarez-Garcia and Miska from the Wellcome Trust Cancer Research,³² reviewed the functions of microRNAs in animal development and evolution. The action of this little molecule in the animal developmental timing, patterning, embryogenesis, organogenesis, cell differentiation, and cell death has been well summarized. MicroRNA family, lin-4 and let-7 as heterochromic genes, have potential roles during evolutionary changes. Many investigators studied microRNAs' roles in evolution.

It functions through the inhibition/inactivation of effective mRNA translation of the target genes. The underlying mechanism and microRNAs target is still unknown, but it relates to their importance in the origin of complexities.^{36,37} Bartel of Massachusetts Institute of Technology (MIT) prepared a list of microRNAs in different creatures from plants to mammals in the hope to find which and when different families of miRNAs in these creatures have emerged.^{3,7}

Researchers discovered miRNA genes interspersed among set of protein encoding genes called HOX clusters. These are revolutionarily conserved molecules. Recent studies have identified new insight into how HOX proteins cause morphologic diversity at the organismal and evolutionary levels. In this occasion miRNAs function as intergenic regulatory transcripts. Cluster of HOX genes, which number from four to 48 per genome—depending on animal—control the patterning and morphogenesis of the main body axis.⁷⁵⁻⁸⁰ Recent evidence shows that HOX genes

are fragmented, become reduced, or expanded in animals that correlate with their morphologic changes in evolution.⁸⁰ miRNAs, involvement in regulating HOX genes of flies and mice is supported by the fact that miR-196, an miRNA, represses HOXB gene expression. These miRNA regions are also conserved across species in the same way as other mechanistic ways that regulate HOX gene expression.⁷⁶⁻⁷⁷ Yekta et al. demonstrated that miR-196 encoded at three locations in mammalian HOX A, B, and C clusters, has extensive cleavage effects to HOXB, C, and D.⁷⁹

MicroRNAs are important molecules in the evolution of the human brain. Recent research showed the comparison between miRNA in human and chimpanzee, and they found about 8% of miRNAs that are expressed in human brain is exclusively related to humans. This finding suggests that evolution is related to the changes in miRNA as much is related for protein-coding mRNA.⁸¹⁻⁸³ Sometimes, RNA carries genetic information, by hitching a lift in the germ-cells independently of DNA. Some investigators suggest that RNA could itself provide an alternative evolutionary structure. The idea that RNA in certain conditions is governed by environmental factors seems very important in natural selection.

In the last decade, much laboratory works have been done to investigate the regulatory and behavioral functions of noncoding RNAs (ncRNAs) in various aspects of development (patterning, embryogenesis, organogenesis, etc.) and their revolutionary roles on evolution on variety of plants and animals. Many of these studies are dealing with the role of microRNAs in different features of animal and plant development. There are several recent studies in the field of microRNA in development and evolution.^{4, 23, 33, 84}

Conclusion

The discovery of the first short-length RNA gene (lin-4, in *C. elegans*) in 1993, and the subsequent numerous scientific reports indicating that many more “RNA of this kind which do not encode protein” exist have announced a new world of RNA that subverts the traditional beliefs about RNA molecule. These small short-length RNAs have a crucial role as regulatory apparatus maintaining all cell activities. MicroRNAs are an abundant class of endogenous nonprotein coding RNA molecules, have been found in plants,

animals, and viruses, and preserved in these creatures; they play crucial roles in gene silencing. This silencing includes translational repression, mRNA cleavage, and mRNA decay initiated by miRNA-directed deadenylation of the target mRNA. These are the main mechanisms of miRNA-guided gene regulation at the post-transcriptional levels.

MicroRNAs have crucial roles in development, developmental timing, cell differentiation, proliferation, programmed cell death, defense against viruses, inflammation, and in pathogenesis of conditions such as cancer, respiratory, mental, and cardiovascular diseases.

RNAi is becoming a new tool in the diagnosis and treatment of diseases and new advances to prediction of the disease outcome. Having this concept, the man-made RNAi manufacturing has got a powerful market place in the interest of pharmaceutical companies.

Finally, now with the advances in new era of RNA activities and recent discoveries, it is likely to have changes in the people’s point of view about how cells regulate themselves, how life becomes more complex, the features of creature developments, how a certain disease develops, how the diseases can be cured or prevented, and even how the process of evolution operates.

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