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MOLECULAR DIAGNOSIS THROUGH THE USE OF MICRO RNA AND ITS IMPACT ON MODERN THERAPEUTICS: A LITERATURE REVIEW

Human Genetics

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ABSTRACT

Advances related to molecular biology are remarkable, from the discovery of the structure of DNA, to the discovery of gene regulatory mechanisms, which includes numerous discoveries of relevant significance. One of the most recent advances includes micro RNA (miRNA), a single-stranded RNA, containing over 22 nucleotides, originating from the transcription of introns or non-coding regions of the genome. In addition to being involved in the etiopathogenesis of multiple diseases, miRNAs can also be used as diagnostic and even predictive tools, potentially opening a new path in medical therapy. This research aimed to describe ways of using miRNA in molecular diagnosis and its impact on modern therapeutics, according to the scientific evidence found. Among its possible uses, the establishment of biomarkers for various pathologies, both diagnostically and for establishing prognosis, has attracted great interest from the scientific community. Their reduced dimensions, as well as their association with proteins, give these molecules great stability in various body fluids, such as blood and urine. In this way, small changes in its body concentration allow the identification of significant changes in correlated processes, both in physiological and pathophysiological processes, such as, for example, the behavior of some tumors. Despite the initial enthusiasm, further research will be needed to find more sensitive and specific markers, in addition to establishing the level of safety of using miRNA in humans.

KEYWORDS

Molecular Diagnosis. Micro RNA. Therapy.

INTRODUCTION

The advances related to the field of molecular biology are remarkable, from the discovery of the structure of DNA to the unveiling of the regulatory mechanisms of genes, constituting several discoveries of a significant nature. One of these recent discoveries includes miRNA. First mentioned in 1990, their regulatory functions have been known since the first decade of the twenty-first century. Studies have shown that these molecules are involved in the pathophysiology of several human diseases, due to their role in the regulation of cell growth and differentiation.[1,2]

The miRNA is composed of a single strand of RNA, containing an average of 22 nucleotides, originating from the transcription of introns or non-coding regions of the genome. More than 3,500 miRNAs have been identified, 695 of which are found in the human species. Given their regulatory role on gene expression, they play a critical role in several stages of intracellular metabolism, such as tissue differentiation, cell cycle, proliferation and apoptosis, as well as protein synthesis, allowing us to infer that defects in this system may be associated with the etiopathogenesis of a wide variety of diseases, such as cancer, a variety of neuropsychiatric disorders, as well as neurodegenerative diseases, heart problems and a myriad of other possible disorders.[3]

Given the importance of these molecules in the control of gene expression and their consequent impact on the homeostasis of organisms, further research is essential to understand the way in which miRNA regulates organic processes, establishing, from there, how structural or functional alterations can contribute to the emergence of pathologies and seek to establish potential ways to use them for the benefit of science, either for diagnosis or therapy, trying to modify the natural course of these diseases.[1,3] The involvement of miRNA in the pathogenesis of multiple diseases opens up an extremely promising universe for the future of therapeutics in medicine.[2]

In this context, understanding the current state of scientific production related to miRNA allows us to understand how research efforts are being conducted in the international scientific community, identify the advances achieved within this area of knowledge, and provide information that allows directing future efforts, identifying new topics and research opportunities. Following this philosophical thought, this research aimed to describe the ways of using miRNA, currently available, for use in molecular diagnosis and its applicability in modern therapy, according to the available scientific evidence found.

Biogenesis and Mechanisms of Action of miRNA

The origin of miRNA begins in the nucleus and is completed in the cytoplasm. It is a process that involves complex reactions, with the participation of numerous enzymes, in addition to various cellular protein complexes, responsible for maintaining the stability of the process as a whole. The end product of this process must be a mature miRNA, capable of performing the intended regulatory function.[2] The steps involve its transcription at the nuclear level, export to the cytoplasm, processing and maturation. Such transcription of miRNA genes, for the most part, is mediated by RNA polymerase II, an enzyme also responsible for the transcription of protein-coding genes.[4]

The first transcript of miRNA, called pri-miRNA, gives rise to secondary structures, which have regions called "stem". In these regions, two RNA segments that have complementary bases pair, and "loop" regions, where the base pairs do not complement each other, generating a circular loop. In addition, the pri-miRNA is processed in the nucleus by a complex containing Drosha and a double-stranded RNA-binding protein, resulting in a structure called a precursor miRNA or pre-miRNA.[5]

Pri-miRNA is exported to the cytoplasm via Exportin-5. When it reaches the cytoplasm, it is processed by Dicer, which will move the handles and thus form an RNA duplex. Subsequently, it is integrated into the RNA-driven silencing complex (RISC), and the two RNA strands are separated, where one of the strands remains attached to the RISC, constituting the mature miRNA, and the other complementary strand ends up suffering degradation.[2,3]

The miRNA and RISC complex (miRISC) regulates gene expression through two mechanisms: mRNA degradation and repression of mRNA translation. Depending on the degree of complementarity between the miRNA and mRNA bases, one of two regulatory pathways can be used: the small interfering RNA (siRNA) pathway and the miRNA pathway. If the complementarity between miRNA and mRNA is nearly perfect, the mRNA will be processed via the siRNA pathway.[4,5]

Thus, the ribonucleases present in RISC catalyze the endonucleolytic cleavage of the mRNA. Although there are regulatory pathways for siRNA in human cells, most human mRNA is processed via the miRNA pathway. The mechanism of action of the miRISC complex has not yet been fully elucidated. Translations can be deleted during or after the translation has started.[3-5]

At the beginning of translation, the association of the ribosomal

subunit 60S with the 40S preinitiation complex may be blocked and death of the polyA tail may occur. Mechanisms after translation initiation include premature dissociation of the ribosome, decreased rate of elongation, and degradation of nascent peptides.[6,7] miRISC complex can also increase transcriptional degradation by recruiting pickling and deadenylation enzymes.[2] The mRNA repressed by miRNA accumulate in cytoplasmic spaces called processing bodies. They are rich in enzymes that promote the removal of dead tissue, the cutting of capsules, or the degradation of mRNA. Although mRNA can also be temporarily stored in these structures. The fragmentation of mRNA in mutant organisms is an important mechanism that controls the translation process.[3,8]

miRNA as a Diagnostic Method and Future Perspectives

Because miRNAs are involved in the gene regulation of many metabolic pathways, the regulation of their biogenesis and degradation is of paramount importance for cellular homeostasis. From the discovery of miRNA to the present, numerous studies have suggested the importance of miRNA in the diagnosis, prognosis, and possibly treatment of various diseases.[8]

Regarding the practical applicability of miRNA, its use as biomarkers for disease diagnosis and prognosis has attracted attention from the scientific community. Its dimensions and correlated proteins give this molecule stability in a variety of body fluids such as blood and urine, variations in its concentration allow results correlated to the patient's clinical condition, especially in cancer.[2,7]

The use of antagomir (anti-miRs), an RNA strand complementary to the target miRNA and capable of producing a blocking effect, has been investigated as a therapeutic measure. iRNA-like particles have the ability to enter cells by inhibiting various specific mRNA. However, such alternatives can have side effects, as antagonists or iRNA can associate with unexpected targets in a partially complementary way, triggering undesirable responses. In addition, these molecules are capable of triggering an immune response with the potential to induce inflammation, resulting in tissue death.[9]

miRNA and Cancer

The importance and role of miRNAs in cancer has been studied since the early 2000s, with studies showing decreased expression of miR-15 and miR-16 in patients with chronic lymphoblastic leukemia. Since then, changes in the expression of several miRNAs in tumor cells have suggested an important link between the dysregulation of these molecules and the process of cancer development.[10]

In theory, all types of tumors exhibit dysregulated expression of some miRNAs. In addition, more than half of the genes encoding human miRNAs have been found to reside in genomic regions that are said to be dysregulated in cancer.[8,9]

The classification of cancer-associated miRNAs is based on the function of oncogenic miRNAs, target mRNA, and tumor suppressors. Mechanisms such as cell migration and invasion, proliferation and apoptosis, which are processes of carcinogenesis, are influenced by the action of these molecules. In most cases, the classification of miRNA is contrary to that of its target and even though they commonly exert an inhibitory function.[11]

The miRNAs, which are fundamental for cell division and/or subsistence, are controlled by the miRNAs that act as tumor suppressors. Oncogenic miRNAs hinder the action of genes that are effective in stopping tumor development, since they have increased action on cancer cells and promoting the division of these cells.[12]

In malignant tumors, it is observed that dysregulations in the miRNA profile express great relevance in the appearance and advancement of cancer,[13,14] in the appearance of metastasis and in cell death rates. Therefore, miRNA analysis can facilitate the identification and diagnosis of tumor subtypes, thus being able to contribute to the prognosis of cancer patients, including in advanced stage neoplasms.[13-15]

miRNA and Cardiovascular Disease

Another highlight about miRNA refers to cardiovascular diseases (CVD), whose spectrum ranges from peripheral arteriopathies, represented by pathologies linked to microcirculation, to coronary artery disease with all its epidemiological impact. It also includes valvular heart diseases, the most diverse cardiomyopathies, all congenital cardiac pathologies, heart rhythm disorders and heart failure, regardless of their etiology.

Despite all the advances achieved in the therapeutic field, CVD still represents one of the main causes of mortality worldwide, which highlights the need for additional studies to expand the level of understanding available regarding the genetic and molecular mechanisms involved in the pathophysiology of CVD.[16,17]

Differences in gene expression, related to miRNA action, play an indispensable role in the determination of cellular metabolic pathways, severely impacting the physiology of organisms. Moreover, miRNAs acting as regulators of gene expression, due to their involvement in several important cellular pathways, can interfere both in the emergence of several CVDs, as well as in the result of the therapeutic strategies proposed for such pathologies.[18]

CVD that occurs due to abnormalities of protein synthesis are invariably dependent on RNA, which is divided into: messenger RNA (mRNA) and non-coding RNA (ncRNA). Depending on their length, ncRNAs can be classified into long ncRNAs (lncRNA) and short ncRNAs, which, despite not being involved in protein synthesis, are related to the regulation of RNA coding.[18]

In this context, lncRNA regulate gene expression through epigenetic, transcriptional and post-transcriptional mechanisms, participating in myocyte hypertrophy and CVD development. In addition, miRNAs control gene expression by degrading or repressing the translation of target mRNA molecules. Finally, endogenous competitive RNAs (ceRNAs) are mRNA regulatory tools, so lncRNAs can interact competitively with miRNA, inhibiting its functions.[16]

Substantial evidence implicates miRNAs in the development of CVD and suggests that they are biomarkers Thus, miRNA has become an active area of research for the development of new diagnostic and therapeutic tools in cardiology.[17,18]

Therapeutic Applicability of miRNA

The therapeutic applicability of miRNA can be fragmented into two important sections. The first of these is the use of miRNA-inhibiting drugs. The second, and no less important, is related to the pharmacological use of miRNA.[1] The therapeutic relevance of miRNA is evident in the treatment of numerous diseases. Several studies have already highlighted the importance of the instability of certain miRNAs in the pathophysiology of numerous diseases.[10]

Within this context, over the past few years, studies on miRNA and cancer have been prominent. The term "oncomiR" was instituted to present the constant binding of several miRNAs with oncogenesis.[11] The action of oncomiR, which are miRNA species, can leverage tumor growth, due to their ability to inhibit tumor suppressor genes.

Studies conducted[13-15], have identified tumors where miR-21 was identified, and in these cases it was verified that oncomiR miR-21 targets tumor suppressor genes such as PTEN13 and PDCD414.[14,15]

It is important to note that a molecule responsible for regulating miR-21 is already being developed by the company Régulus, together with the Sanofi-Aventis industry. These companies are also involved in research aimed at verifying the relevance of this miRNA. The inhibitory action of this modulator is due to the restoration of the expression of PPAR-alpha15 and Mpv17 proteins, established as miR21 targets.[1,12]

RG-101 is another substance created by Régulus in partnership with Ionis Pharmaceuticals and GSK, which also focuses on miR-122 in hepatocytes infected with the Hepatitis C virus.[14] RG-012 is an anti-miRNA against miR-21, which was also evaluated by the company Régulus in partnership with Genzyme (Sanofi). This drug works by reducing the fibrogenesis of organs associated with Alport syndrome. miR155, a miRNA involved in the differentiation and proliferation of blood and lymphoid cells, also has an antagomiR under development by MiRagen Therapeutics, which is producing MRG-106, also called Cobomars.[13]

MRX34 has shown promising results in preclinical studies when used against a variety of cancers such as renal cell carcinoma, acral melanoma, and hepatocellular carcinoma.[15] However, the phase 1 clinical trial was interrupted after the occurrence of serious adverse events related to the immune system, including death.[13,15]

MiRagen Therapeutics has developed MRG-201, also known as

Remlarsen, which mimics miRNA that aims to restore levels of miR29b, a negative regulator of the extracellular matrix. Phase II clinical trials are underway and, to date, the drug has shown promising potential in the treatment or prevention of pathological fibrosis of the skin, as well as other fibrotic diseases, such as idiopathic pulmonary fibrosis.[10.12]

CONCLUSIONS

In view of this evidence, we can conclude that the human genome is composed of several miRNAs, however, its true physiological functions and pathological implications are not yet fully elucidated, due to being a recently addressed topic. New discoveries allow for numerous practical applications in various sectors. A number of diagnostic methods based on these molecules have already been identified. Several therapeutic methods are being tested on animals.

Despite promising advances in health, more research is still needed so that we can discover more sensitive markers with greater specificity. Likewise, it is necessary to conduct studies that produce sufficiently robust scientific evidence, capable of establishing the safety of the use of miRNA in humans, serving as support for the advancement of research in this direction. The great potential of these substances on the future of human health care is undeniable, and it is up to experts in this area to broaden their horizons on miRNA-related technologies, keeping up to date with the latest research and assets in terms of expanding the restricted database available on this topic.

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