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Review Article

MiRNAs: regulators of human disease

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Summary

MicroRNAs (miRNAs) represent the mediators of important leading biological functions of molecular pathways in humans. They are a class of very small, non-coding RNAs; their function is the balance of the protein levels at the post-transcriptional stage. They are implicated in molecular processes and diseases, including diabetes, metabolism, autoimmune diseases, angiogenesis and tumorigenesis, and female fertility, exhibiting an altered expression profile. Any process taking place in the human organism is intertwined by miRNAs. MiRNAs have an impact on the biochemistry of pathways of the invisible molecular world. They circulate in a stable chemical configuration in body fluids (tears, serum, plasma, amniotic fluid, ascetic fluid, urine) with their molecular sequence specificity remaining unchanged. Their indisputable molecular stability ranks them as extremely vigorous potential markers in human disease. MiRNAs demonstrate a specific expressive signature, representative of the tissue specificity and the clinical staging. The shift on the concentration and expression of a miRNA reflects the course of a disease. MiRNAs may operate as oncogenes (tumor growth) or tumor suppressor (tumor reduction) genes in cancer pathways. In malignant disease, proliferation, maintenance, and progression of cancer cells is induced by the stimulation of the oncogenes or complete deactivation of the tumor suppressor gene activity.

Key words: Cancer diagnosis; miRNA expression; Endometrial cancer; miRNA.

Introduction

MicroRNAs (miRNAs) have been designated as the universal regulators of genome expression demonstrating either onco-suppressive or onco-inducing potential. They are very small RNA molecules with an approximate length of 19-22 nucleotides; they discreetly regulate gene expression through the RNA interference mechanism (RNAi). RNAi is the term used for the post-transcriptional mechanism detected in eukaryotes; it targets the double-stranded RNA (ds-RNA) and triggers the degradation of the messenger RNAs (mRNAs) in a sequence specific mode. The short interfering siRNAs function as the mediating molecules originating from the exogenously presented ds-RNA. MiRNA genes are distributed along the chromosomes either as individual genes or assembled in small units. MiRNAs demonstrate a limited and partial complementarity against the targeted sequences. They demonstrate strict tissue specific and growth expression patterns. In cancer, they show particular expression patterns that correlate with clinical features. Cell proliferation and differentiation, apoptosis, regulation of insulin secretion, and blood lineage differentiation processes constitute some of the vital processes that take place in organisms and are being regulated by miRNAs; these very small molecules compose parts of the biochemical network in the cell. The aberrant function of a miRNA can disturb cellular pathways and consequently open the pathway to a disease [1-3].

Structure and biogenesis of microRNAs

MiRNAs are a family of small regulatory non-protein coding RNAs with short sequences of 19-22 nucleotides. Their structure is made up of nucleotides, with three components: a nitrogen-containing base, a five-carbon sugar (pentose), and a phosphate group linked to the pentose by a phosphodiester bond.

The nucleus and the cytoplasm are the two cellular regions where miRNA biosynthesis takes place. Everything starts with the formation of a large hairpin structure from the initial miRNA transcript. A nuclear located microprocessor complex consisting of the Drosha RNAase III endonuclease and the double-stranded-RNA-binding protein DGCR8 (in the human organism) or the Pasha species (in Caenorhabditis elegans and in Drosophila melanogaster), splits the pri-miRNA transcript at the base causing the release of the novel pri-miRNA stem-loop molecule. Exportin-5, a nuclear export protein, functions as the active transporter of the pre-miRNA into the cytoplasm where additional processing by the cytoplasmic Dicer RNAase III endonuclease is carried out. The action of Dicer on the pri-miRNA releases the newly formed

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double-structured miRNA. The RNA-induced silencing complex (RISC) is a multiprotein structure that incorporates protein molecules such as the Argonaute and assists in the attachment of the miRNA to its target genes. One strand is removed and degraded, the second strand of the miRNA configuration is trapped by the RISC complex which in turn aims at mRNAs; the sequence specific complementarity determines the translational repression or the direct degradation [1-3].

miRNAs in human diseases

miRNAs in cardiovascular diseases

Different levels of miRNAs are associated with different diseases. Clinical studies have demonstrated that miRs acquire a fundamental role on cardiovascular diseases. MiRs are expressed and circulate in the blood stream; their presence and circulation give the opportunity to control the fluctuations and stability of the cardiovascular system. A group of miRs (miR-126, miR-145, and miR-155) have been identified in a down-regulative mode in coronary arterial disease (CAD) patients, while others (miR-208a) circulate in an up regulated mode. The miR-21/590-5p family, miR-17/92a cluster and miR-126, are part of a circulating miRNA signature and represent a potential marker for the detection of candidate CAD patients.

Acute myocardial infarction (AMI) comprises cardiac-related miRs, i.e., miR-208, miR-1; cardiac-specific (miR-208), muscle-enriched miRs (miR-499, miR-663b, miR-1291), and smooth muscle-enriched (miR-145) have been identified as potential markers for AMI [5-7]. Patients with AMI demonstrate increased levels of the miR-208b and -499 and show a correlation with detectable troponin concentration. The miR-423-5p subtype has been detected in heart failure patients. Moreover plasma concentration for miR-1 is increased in AMI subjects [4-7]. Based on a clinical study, AMI is related to a decreased miR-1 concentration [8]. Stillness of miR-208a leads to enhanced heart function and increased survival rate in heart failure (HF) rat models. In rat models with a mean cerebral artery occlusion (MCAO) syndrome, blood-related miRs (miR-215, miR-324-3p, miR-451, and miR-134) were increased in blood circulation. It seems though that miR modulation can have applications on neurobiology. Specifically, regulation of miR-497 can restrict infarct spreading and enhance neural cells. MiR-181, a potent modulator of Bcl-2 and Mcl-1 eliminates extended brain infarcts [5-9].

MiRNAs in cancer

Overexpression of miR-21, -92, -93, -126, and -29a was detected in ovarian cancer patients, and miR-155, -99b, and -127 were underexpressed. In a sample of breast cancer subjects, miR-10b and -34a were identified. MiR-195 and -7a were observed in reduced expression levels in patients with ovarian neoplasms [10-13]. MiR-155 in humans is detected in increased levels in acute leukemia and lymphomas; in acute myeloid leukemia patients, miR-196b is upregulated. Furthermore, the underexpressed miR-223 is found in ovarian cancer [14, 15].

MiR-125 may function as the cancer mediating factor in type II EC. MiR-125b-1 and miR-125b-2 illustrate a higher prevalence rate in Stages III and IV in endometrial carcinomas (ECs) compared to Stage I endometrioid ECs. Other studies suggest that miR-103, miR-205, and miR-210, are upregulated in EC and miR-205 and miR-182 are involved in tumor lymph node metastases [16-19]. These observations suggest that there are miRs involved in tumorigenesis initiation and miRs responsible for tumor growing and reoccurrence.

Lung cancer development seems to be controlled by miRNA let-7. In lung malignancy, the titer of let-7 is decreased and this is related to postoperative progression. Cell growth is induced by miR-17-92, which is exceptionally increased in small-cell lung cancer, ovarian cancer, two of the most aggressive neoplasms [17-19].

miRNAs in Alzheimer's disease

Alzheimer's disease (AD) is the most commonly encountered neurological disorder characterized by a slow development. The circulating let-7b miR can deteriorate brain function through binding to the Toll-like receptor 7 (TLR-7); TLR-7 is a highly conserved protein expressed in lung and placental tissue, involved in pathogen recognition and activation of innate immunity [20-25]. Expression of miR-9 in the hippocampal region of AD subjects has been described [24].

Cerebrospinal fluid (CSF) circulates in the brain ventricles, functions as a lubricating means of the brain, and within the spinal cord canal. In the CSF samples of 13 AD patients, significant concentration of the let-7b was detected. The pro-inflammatory subtypes miR-9, -125b, -146a, and -155 were calculated in CSF samples of AD patients, with the use of fluorescence-based miRNA microarrays [20-23]. Geekiyanage *et al.* demonstrated a reduced level on miR-181c, miR-9, miR-137, and miR-29a/b in control and AD patients by using the method of qRT-PCR [21].

Peripheral blood mononuclear cells (PBMC) are vital components of the immune system, to fight infection and adapt to intruders; Bhatnagar *et al.* provided evidence that the miR-34c circulates in abundant concentrations in PBMC and the plasma part of AD patients [23-25].

The variable expression of microRNAs in human disorders

In a healthy organism, miRNAs are the global modulators of pathways of gene expression. They function in a blocking mode; they inhibit the expression of target genes by binding to complementary regions in mRNAs. MiRNAs

cause the degradation of target mRNAs or alternatively prevent the translation of target mRNAs. MiRNAs monitor and coordinate basic cellular processes in eukaryotes [1-3]. MiRNAs are the sensitive sensors of the physiological changes along the biochemical pathways. They do not encode for any protein products; they regulate the expression of target mRNAs, with numerous miRNAs targeting a single mRNA. There is a number of mechanisms (physical deletion, overexpression, and epigenetic silencing) leading to either inhibition of an anti-oncogenic pathway or to strengthening of an oncogenic pathway [26-30]. Gene expression in human cells is regulated in an advanced mode through complex gene regulatory molecular networks. MiRs are short length molecules and the high sequence similarity between closely related microRNAs makes it hard to detect them with sufficient specificity and sensitivity. Due to their short length, any interaction between a miR and any other molecule is exceptionally stable 30-34.

Dysfunction of miRNA expression can occur through a variety of processes, i.e., abnormal miRNA processing, chromosomal rearrangements, promoter methylation modifications, and transcriptional regulators, processes that occur in human neoplasms [35-36]. Alterations in miRNA expression can affect vital biological pathways in cell multiplication, apoptosis, cell differentiation, malignancy, and metabolic balance, by suppressing the expression of their target genes; any alterations on the behavior of a miRNA might notably affect the functional ability of other genes [36-40]. The difference in expression levels can be used to differentiate between malignant and healthy tissues and decide on the clinical course. The tissues' developmental pathways are modulated by miRNA expression. MiRNAs can be used efficiently as unique innovative markers to standardize tissues; the range of miRNAs and their accompanying gene indicate the tumor histological type [40-44].

MiRNAs act as a fine switch and a modulator of gene expression. Each miRNA is directed at one gene; complete silencing of the target genes by miRNAs, occurs through either translation modulation or deactivation of the target messenger RNA (mRNA), and eventually impeding protein synthesis.

More than 1,500 human miRNAs have been deciphered so far. MiRNAs seem to function as the primary regulators of human carcinogenesis; a great number of miRNAs is down-modulated in neoplasia [24]. A single miRNA has the ability to interfere in the expression of a broad network of genes in normal cell function. As a miRNA regulates the function of several miRNAs, it accurately coordinates the biological pathway which can genuinely affect several cellular activities. Loss of continuity and coherence in miRNA biosynthesis and function may lead to disease. Normally when a miRNA is deregulated, the pathways that are the targets of this specific microRNA are being disorganized; the delicate balance and the order of events in normal cell function are disrupted.

The deregulation of miRNAs can have a negative impact on normal gene expression and play a role on the initiation, progression, and maintenance of human diseases, such as cancer, fibrosis, and cardiovascular diseases [5-9]. Aberrant expression of miRNAs is observed in most tumor types, denoting a role in carcinogenesis and a potential involvement as a prognostic biomarker in malignancy. Cancer progression has been related to the expression profile of different miRNAs [10-12].

MiR-15 and miR-16 were the first ones to be identified in tumor samples from B-cell chronic lymphocytic leukemia (B-CLL). Both miRNAs were deleted and downregulated in B-CLL [15,35]. The let-7 protein group was elucidated on non-small lung cancer showing a tumor suppressive activity [18, 19, 36].

Circulating miRNAs offer great hope for the diagnosis and possibly prediction of cancer. In terms of biological and cellular functions, very few things have been revealed of the discovered miRNAs so far. Circulating miRNAs in serum and plasma reflect tissue changes, organ injury, and disease states.

Myatt *et al.* reported that the apoptosis-associated genes are negatively induced by the abnormally expressed miRNAs (miR-9, miR-27, miR-96, miR-153, miR-182, miR-miR-183, and miR-186) and play a decisive role in endometrial cancer expansion [37]. Aberrant expression of miR-21 has been connected to clinical staging, lymph node occurrence, and poor clinical outcome in breast cancer (BC) patients. An upregulation pattern of miR-21 was detected in subjects with significant metastatic potential [40-41]. The tropomyosin (TPM) protein, a component of the microfilaments in the cytoskeleton, is destabilized in tumor cells; the tumor suppressor gene tropomyosin 1 (TPM-1) is related to tumor spreading. Except for TPM-1, programmed cell death 4 (Pdcd4) and mammary serine protease inhibitor (maspin) promote cell proliferation and tumor metastasis; these genes have been identified as potential targets for miR-21. The Her2/neu (receptor tyrosine kinase) positive cells exert a downregulative effect on Pdcd4, while miR-21 is upregulated through a triggering effect originating from the Her2/neu cellular cluster. Apoptosis of the cells is inhibited by the oncogenic mechanism of miR-21; miR-21 is involved in cell multiplication, inflammation, metabolism, angiogenesis, and tumorigenesis [41-45]. Furthermore, miR-21 aims at specific tumor suppressor genes, such as Pdcd4, TPM-1, and sprouty 2 (SPRY-2), and prevents them from releasing their tumor deactivating power. MiR-21 has been detected in endometrial endometrioid cancer (EEC) cells to be upregulated in comparison to normal endometrial tissue. Up-modulation of miR-21 promotes cell division and down-modulation of miR-21 has the opposite effect on the cell cycle [41, 46-48].

A common gynecological tumor is EC; it is divided into two main groups, type I and type II, with its mechanism of

progression remaining unsolved. Type I is estrogen related, of good differentiation and prognosis, and low grade. Type II, on the other hand, is devoid of estrogen receptors, and demonstrates low differentiation, high grading, and poor prognosis. Type II EC is not so common (15% of cases) compared to type I EC cells (85% of cases) [41, 42]. Clinical studies have shown that miR-125-b is upregulated; it is the major inducer of uncontrolled cell division in type II EC. MiR-125-b, a sequence specific regulator, exerts its function through its target suppressor gene, the tumor protein 53-induced nuclear protein-1 (TP53-INP-1) [16, 41-42]].

MiRNAs target, annihilate and induce the translational repression of their target mRNAs [49-53]. MiR-103 promotes tumor development and metastasis in endometrial cancer cells. MiR-103 is overexpressed in an irregular mode and deactivates its tumor suppressor gene, the tissue inhibitor metalloproteinase-3 (TIMP-3) from blocking cell proliferation in endometrial tissue [54-59].

Two types of genes regulate cell division; the oncogenes (aggressive type) originating from proto-oncogenes and the tumor suppressor genes (protective type). Only a few in number have with certainty been identified as either oncogenic or tumor suppressive molecules. A mutated tumor suppressor gene might lose its breaking power; the result is uncontrolled cell division [60-63]. The oncomiRs are oncogenic miRNAs that induce tumor development; miR-155 is one example of an oncogene miR. In pediatric oncology, miR-155 is overexpressed in Burkitt's lymphoma and in aggressive chronic lymphocytic leukemia. The same genetic transformation with large B-cells going through a polyclonal expansion and developing all high-grade lymphoma has been observed in transgenic mice [45-49].

One of the major challenges is the extent of the miRNA involvement in female biological processes. The miRNAs are profound molecular regulators of gene expression and an essential component for the normal development of the female genital tract; they dynamically participate in cell multiplication and differentiation in female fertility in a variety of diseases and neoplasia [49-53]. Ovaries, fallopian tubes, cervix, and uterus are the major anatomical parts that compose the female genital tract. Appropriate development and function of the component parts of the tract are the two main prerequisites for a successful reproductive process [49-53]. Otsuka *et al.* experimented on mice mutants with the Dicer 1 allele and proved that lack of the allele can lead to female infertility. The inactive state of Dicer 1 expression may lead to luteal insufficiency and an unsuccessful gestation [48-52]. Absence of miR-17-5p and let7b-regulators of the tissue inhibitor metalloproteinases-1 (TIMP-1) factor was related to these deficiencies [38]. Abnormalities of the derived from the Müllerian ducts' tissues (short uterine horns and cysts on the oviducts), were encountered in female mice with the Dicer I enzyme in a dormant state [49]. MiRNA expression profile is under clarification in women with ovarian cancer, one of the most common malignancies in the female genital tract [49-51]. Clinical investigation focuses on the miRNA expression between cancerous and normal tissues; it is of major importance the identification of the miRNA signatures in tissue lines [64-68]. There are diseases in which the exact role of miRNAs is completely unraveled [40, 59]. The expression of miRNAs has been studied in uterine neoplasms, either benign or malignant. Boren *et al.* compared normal endometrial tissue samples with adenocarcinomas and revealed 13 differentially expressed miRNAs (miR-let-7i, miR-221, miR-30c, miR-152, miR-193, miR-185, miR-106a, miR-181a, miR-210, miR-423, miR-103, miR-107, and miR-let-7c) to be associated with endometrial tumor growth [55]. Differences on the mRNA level were observed between normal tissue and uterine adenocarcinomas; the 9% of the mRNA were actual targets of the 13 differentially expressed miRNA [55, 56]. Uterine leiomyomas is a type of benign neoplasms in gynecologic oncology. Microarrays were used for miRNA detection in normal endometrium and uterine leiomyomas. A clinical study was conducted and uncovered 81 miRNA species being expressed. The antiapoptotic miR-21 and the pancreatic adenocarcinoma indicator miR-139 were among the identified subtypes [56, 68-70].

Virology and molecular biology seem to be interconnected; chromosome 13 hosts the miR-17 cluster, a miRNA amplified in B-cell lymphomas. The miR-17 in concertion with c-Myc accelerates tumor progression in mice B-cell lymphomas. The miR-17 genome group functions by hindering programmed cell death [71-73]. The Epstein-Barr virus, a member of the herpes virus family, encrypts a number of miRNAs with an unknown function as of yet (miR-S1). Furthermore, miR-32 regulates the multiplication of the primate foamy virus type-1 (PFV-1) [44, 45].

Conclusions

MiRNAs are non-coding RNAs with a multifunctional role in human disease; a great number has been identified and connected to diseases [70-72]. It seems though that other non-coding RNAs are still to be discovered. MiRNAs have evolved as stable biomarkers demonstrating their potential in guiding biochemical pathways to a precise direction; they serve as signaling molecules in intercellular communication. There are classes of non-coding RNAs that contribute to miRNA quantification, tumorigenesis, and ribosome synthesis. Clinical research is still at the beginning; microRNAs open a new horizon in disease identification [66, 74-76]. MiRNAs possess the capacity of a diagnostic and prognostic means, in treatment decision, and disease progression in medical science. Serum biomarkers permit the analysis and investigation of cardiovascular, neurodegenerative diseases and neoplasms without the use of invasive procedures [5-7, 11-14, 20-23].

Their surprising stability over time in biological fluids

has designated them as biomarkers in human neoplasia, neurological diseases, and cardiovascular diseases. It is of note though that messenger RNA (mRNA) is spontaneously degraded in plasma, while miRs are protected from the circulating RNAase enzymes in blood. Their molecular stability is illustrated by mechanisms; miRs are either encapsulated in structures named exosomes, or linked to plasma proteins. Programmed death cell and cellular disintegration of cancer cells result in their release in the bloodstream [77-80].

An alteration on the miR expression takes place earlier than ordinary markers. Chemokines and cytokines, the current markers of inflammation, are detectable when a large part of damage has occurred. It is likely that miRs may reflect the current state of molecules and tissues and act as intermediaries in cell-to-cell interaction and signal transduction; it is noteworthy that more accurate and sensitive markers should be revealed for the immediate detection of human diseases [81-87].

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