Artículo de revisión

MicroRNA: biogenesis, functions and objetives identification

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RESUMEN

Los microRNA son moléculas de RNA de 20-30 nucleótidos no codificados de una sola cadena capaces de regular la expresión genética en organismos eucariotes. Muchos estudios han demostrado la participación de los miRNAs en el cáncer, por una variedad de mecanismos, como: la amplificación, deleción, mutaciones y factores epigenéticos. Esta revisión resalta su importancia, diferencias y mecanismos de validación.
Palabras clave: RNA, microRNA, biogénesis, funciones y objetivos de identificación, cáncer, genes supresores tumorales, oncogenes, regulación genética.

ABSTRACT

MicroRNAs are non-coding 20-30 nucleotide single stranded RNA molecules that are capable of regulating gene expression in eukaryotic organisms. Several studies have shown the involvement of miRNAs in cancer by a variety of mechanisms such as amplification, deletion, mutations and epigenetic factors. This review outline their importance, differences and validation mechanisms.
Key words: RNA, microRNA, biogénesis, funciones y objetivos de identificación, cancer, tumor suppressor genes, oncogenes, gene regulation

The study of small RNA molecules such as microRNA (miRNA), short interfering RNA (siRNA) and the process known as interference RNA (RNAi) started with the identification and interaction of lin-4, a small non-coding 22nt length RNA and its major target lin-14 as an endogenous gene regulator in C. elegans.[1,2] Alongside this discovery, the finding of double-stranded RNA molecules with gene silencing effects continue to expand the research in molecular biology with still upcoming new applications in diverse areas related to eukaryotic organisms.[3]

Both miRNA and siRNA have similar characteristics involving their molecular structure, formation and effect pathways. However, they have important differences, miRNAs were seen as endogenous post-transcriptional mechanisms of an organism genome, while siRNA were proposed to have an exogenous origin, also differing in their target recognition and silencing mechanisms.[4-7] Up to date, miRNAs and their regulation effects have been identified in plants, flies, worms and their counterparts in 6,8-10 mammals.

Due to their special location inside or close to fragile sites, of loss of heterozigosity and minimal regions of amplification11, miRNAs are constantly studied as targets in cancer research. Also, they are involved in other biological processes such as metabolism and disease due to their regulatory effect in almost up to 30% of human 6,12,1314-17 genes in apoptosis, differentiation and cell proliferation.

This review focuses on the structural differences between miRNA and siRNA, their biological functions, examples of them as models of oncogenes or tumor suppressor genes and finally their validation tests to confirm new miRNAs prospects.

Biogenesis and structure of miRNA

The first step in the biogenesis of microRNA is pri-miRNA, and comes mediated by a RNA polymerase II transcription with a double stranded stem of 33bp, a

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terminal loop and two single-stranded segments18. After transcription, the pri-miRNA is cleaved by the microprocessor complex into a 70 nt length hairpin-like precursor of miRNA (premiRNA).

The microprocessor consists of a RNase III enzyme Drosha and a binding[19,20] protein DGCR8/Parsha.This protein binds to the hairpin structure base, the stem, and positions the enzyme Drosha that cleaves the stem of the pri-miRNA from the two single stranded segments.[21] Other proteins involved in this process are p68, p7222 and a Drosha independent pathway has also been described.[23] Pre-miRNA is then exported to the cytoplasm from the nucleus by Exportin-5 and the RanGTP hydrolization to RanGDP6.[24] Once the pre-miRNA is in the cytoplasm a RNase III enzyme with endonuclease activity called Dicer, dices the pre-miRNA into short RNA duplexes.[25] Mature RNA duplexes consist of a mature miRNA strand and a complementary miRNA strand depicted as miRNA* that undergoes degradation.[26]

Mature miRNA binds to an Argonaute (Ago) protein forming the so called RNAinduced silencing complex, RISC.[27] The Argonaute superfamily can be divided in diverse subgroups: Piwi, that binds to piRNAs, a nematode specific clade and the Ago clade that associates with miRNAs and siRNAs,[28] they have 4 characteristic domains:[29,30]

a Dicer shared PAZ domain, PIWI, N and Mid. MiRNAs mediate target recognition in different patterns, while there is almost a perfect complementarity in plants, in most animals there is multiple imperfect pairing.[31] Recent experiments have determined that the most important factor in target RNA recognition by a miRNA is perfect to almost perfect pairing of the 5' region consisting of 2-8 nucleotides of the[32,33] mi-RNA and the mRNA, this region is called seed or nucleus.

Between the theories explaining the mechanisms by which miRNAs mediate gene silencing three will be discussed in this review: miRNA mediated translational repression, miRNA-mediated degradation, and P-bodies. MiRNA-mediated translational repression is a post-transcriptional mechanism of gene silencing in which miRNA targets exhibit significant down-regulation at the protein level.[34] MiRNA mediated mRNA degradation occurs by deadenylation and/or decapping through recruitment of GW182 via Ago-mediated interaction.[35] The segregation of miRNA function into cytoplasmatic foci containing a number of molecular machinery involved in mRNA degradation pathways is the basis of the P-body or GW-body theory,[36] still, the formation of P-bodies does not always precede silencing and there is still debate about this mechanism of repression.[37]

Biogenesis of siRNA SiRNA starts as a long linear, perfectly based paired dsRNA; these structures are then processed by DICER like miRNA biogenesis, into siRNAs. Although single stranded siRNA can load into Ago proteins, the human dsRNA depend of mechanisms to attach to it. SiRNA were thought to have an exogenous origin, since they were seen in transgene and virus induced silencing in plants3 however they also have endogenous sources like convergent mRNA transcripts, hairpinRNA (hpRNA) and sense antisense, pairs.[38,39] RISC assembly has been characterized in Drosophila, and appears to be biochemically simpler in humans and is mediated by the involvement of three proteins: DICER, TRBP, and Ago220, even additional proteins are associated with Ago complexes in human cells but does not seem to be essential for RISC loading or target cleavage.[40] There are different RISC assembly pathways that dictate thus, different categories of siRNAs, this finding applies depending the endogenous or exogenous nature of the siRNas.[38,39,41,42]

RNA degradation is induced by the PIWI domain of the Ago protein, with a precise cut in the phosphodiester links of the targeted nucleotides, resulting in products with 5' monophosphate and 3' hydroxyl termini,[39] this process is then finished by cellular exonucleases that degrade the resulting fragments.[43] As with miRNAs, the effector phases in siRNAs occur mainly in cytoplasmatic locations known as P-bodies (GWbodies) that exhibit high amounts of mRNA factors.[44] A noteworthy effect of siRNA is its ability to amplify its effects, causing a striking response that may lead to systemic silencing spreading through an organism3,40. Interestingly also is the fact that siRNA is involved into inducing heterochromatin formation in S. pombe.[45,46]

One of the key differences between miRNAs and most siRNAs is the precision of their ending sequences, while miRNA have highly exact ends while siRNA are more heterogeneous, this gives higher specificity to miRNAs on substrate.[21]

Oncologic associated studies involving miRNAs are based on their profile expression between normal cells and cancer cells; this expression is highly specific for cell-type and differentiation status. However, care must

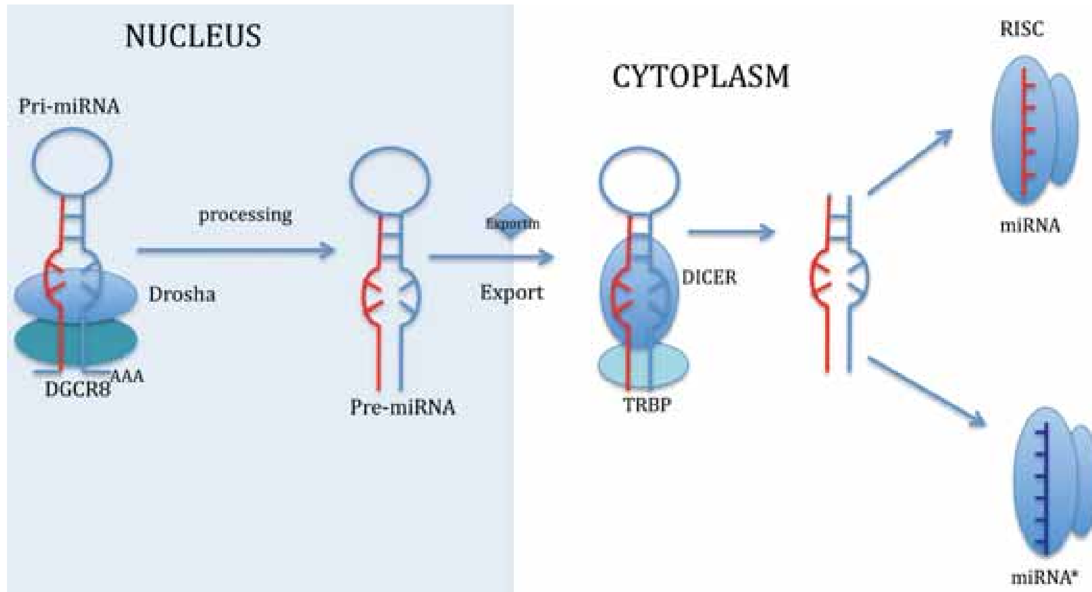

Figure 1. Simplified biogenesis of mi-RNA Small Interfering RNA (siRNA)

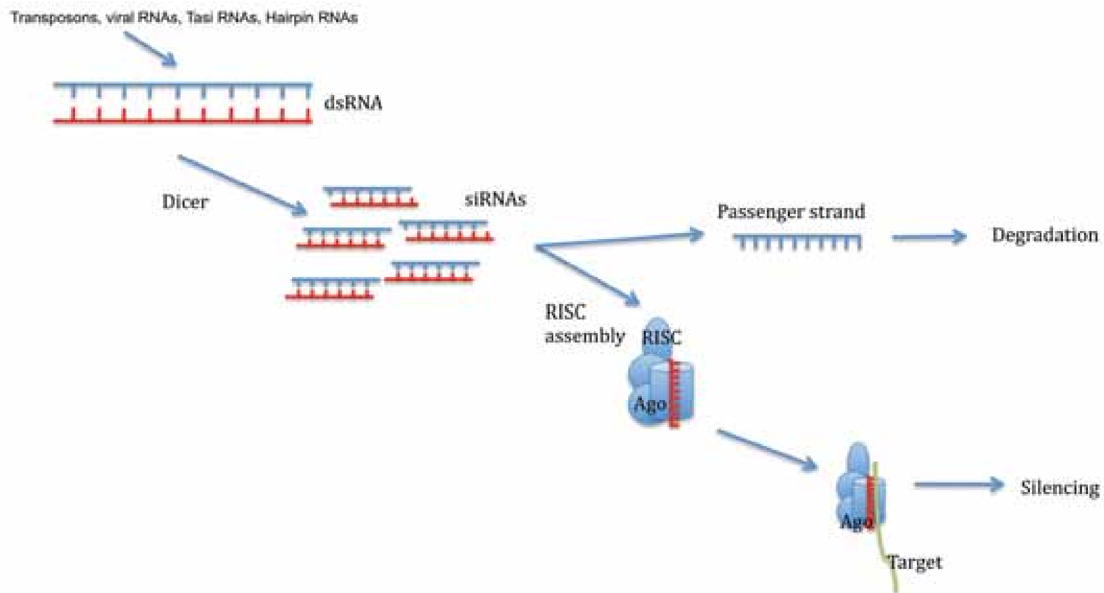

Figure 2. Simplified biogenesis of siRNA and its effects.

be taken since tumors may arise the formation of aberrant miRNA expression as a consequence of the malignant transformation / mutations involved in the cell. Evidence that miRNA can have tumor suppressor or oncogenic activity should be related to: 1) Data demonstrating widespread dysregulation in diverse cancers, 2) Gain or loss of miRNA function in tumors owing to deletion, amplification or mutation, 3) Direct documentation of tumor-suppressing or tumor-promoting activity using animal models and 4) Identification and verification of cancer-relevant targets that clarifies mechanisms through which the miRNA is involved in the oncogenesis.[47]

Oncogenic MicroRNA examples

MicroRNAs whose expression is increased in tumors may be considered oncogenes (oncomirs) and have the ability to promote tumor development by inhibiting tumor suppressor genes, genes that control apoptosis or genes involved in controlling cell differentiation. Some examples that will be discussed are mir-17-92, BIC/mir-155, mir-21 and mir-372/373.

Mir-17 cluster involves six human miRNAs (mir-17-5p, mir-18a, mir-19a, mir-20a, mir-19b1 and mir-19b2) located in chromosome 13q31-32, this genomic locus is highly expressed in solid tumors and hematological malignancies such as large B cell lymphoma, mantle cell lymphoma, primary cutaneous B cell lymphoma, colon, lung,[48-52]prostate, breast, stomach and pancreatic cancers. The expression of the mir-17 cluster oncomirs is related to the consecutive expression of the c-Myc gene, which regulates the expression of E2F1 gene involved in cell cycle. Mir-17-5p and mir-20a repress E2F1 translation, mutants of mir-20a causes a four-fold increase E2F1, thus the regulation of c-myc by mir-17-92 modulates the expression of E2F1 affecting cell[52,53] death via the ARE-p53 pathway. This came as evidence by in vivo using E•-myc in transgenic mice. Hematopoietic stem cells from E•-myc animals formed B cell lymphomas when introduced to lethally irradiated recipient animals,[54] this alongside another study demonstrating that expression of the mir-17 cluster increased the proliferation of lung cell cancer in vitro.[49]

Mir-155 is embedded in the B cell integration cluster (BIC) located in chromosome 21q23, and is highly expressed in pediatric Burkitt lymphoma, Hodgkin disease, primary mediastinal non-Hodgkin lymphoma, CLL, AML, lung cancer and breast cancer.[50,55-59] Coexpression of BIC and c-myc is related to cause growth enhancement of cells,[57,60,61] in AML is correlated with higher counts and tandem mutations and in early leukemogenesis it showed polyclonal preleukemic pre-B cell proliferation followed by malignancy transformation in a mouse model with a B cell– overexpression of mir-155.[60,62]

Mir-21 functions as an oncomir in glioblastoma, it was discovered while screening with expression arrays and northern blot showing its abnormal miRNA expression. It is upregulated in human glioblastoma tissues, primary tumors, and glioblastoma cell lines related to adult and fetal brain tissue and astrocytes. Studies show that mir-21 may promote tumorigenesis by inhibiting apoptosis via inactivation of caspases.[63]

Besides its involvement in glioblastoma, mir-21 has been found upregulated in hematological malignancies and solid tumors and has been shown to inhibit cell growth in cultured liver and breast cells.[65]

Mir-372 and mir-373 participate in the oncogenesis of human testicular germ cell tumors; this was assessed after monitoring CDK2 activity in miRNA expressing cell lines.[66]

Induction of p21 following Ras activation did not inhibit CDK2 in the presence of activated Ras when mir-372 and mir-373 was expressed. The microarray analysis revealed down regulation of large tumor suppressor homolog 2 (LATS2)67, which acts as an inhibitor of CDK2, relieving CDK2 from repression and promoting proliferation in the presence of activated Ras.[66]

Table 1. Examples of Oncogene activity of miRNAs

MicroRNA Disease involved Effects
Mir-155 CLL, DLBCL,
AML, BL, lung and breast cancers
Induces lymphoproliferation.
Mir-17-92 Breast, lung, colon, stomach, pancreatic tumors and lymphomas.
Induces lymphoma and lymphoproliferative disorders
Mir-21 Breast, colon, pancreas, prostate, lung, liver, glioblastoma.
Induces apoptosis and decreases of tumor formation Mir-372/373
Testicular tumors Promotes tumorigenesis alongside with RAS
MicroRNAs as tumor suppressor genes.

MiRNAs whose expression is decreased in cancer cells and usually prevent tumor development by negatively inhibiting oncogenes or the malignant transformation of cells, are regarded as tumor suppressor genes. This loss of function can be due to several mechanisms such as genomic deletion, mutations, epigenetic silencing and miRNA processing alterations.[68,69]

B cell lymphocytic leukemia is the most common leukemia in adults in the western world, is characterized as a monoclonal disorder with progressive accumulation of incompetent lymphocytes. The cells of origin in the majority of patients with chronic lymphocytic leukemia (chronic lymphoid leukemia, CLL) are clonal B cells arrested in the B-cell differentiation pathway, intermediate between pre-B cells and mature B cells. Morphologically in the peripheral blood, these cells resemble mature lymphocytes.[68] An abnormal karyotype is observed in the

majority of patients with chronic lymphocytic leukemia (chronic lymphoid leukemia, CLL). The most common abnormality is deletion of 13q, which occurs in more than 50% of patients.

Individuals showing 13q14 abnormalities have a relatively benign disease that usually manifests as stable or slowly progressive isolated lymphocytosis.[70,71] Until recently, a cluster of miRNAs, mir-15a and mir-16-1, was discovered in the 13q14.2 region. This cluster was shown to be deleted or down regulated when compared to normal CD5+ lymphocytes from normal donors. The tumor suppressor role in this cluster was made by the discovery of the upregulation of the antiapoptotic bcl2 gene. Deletions of miRNA15a and miRNA16-1 lead to overexpression of bcl2 through loss of down regulating miRNAs. [72,73] miRNA16-1 also plays a critical role in the recognition and rapid degradation of transcripts containing AU-rich elements, although its pathologic relevance still needs further research.[74,76]

Let-7 was originally identified in C. elegans, and its loss of function prevents the transition from the fourth larval phase to adult cell fates in the worm.[17] This gene is highly conserved in vertebrate organisms from worms to humans.[77-79]

Loss of expression of let-7 results in loss of differentiation, and members of this gene functionally inhibit the miRNAs of genes such as Ras family genes, HMGA2and c-myc81, inducing programmed cell death when is over expressed in lung cancer,[79,81] colon cancer and Burkitt lymphoma cell linesThe mir-29 family includes three isoforms in two clusters: mir-29b-1/mir-29a and mir-29b-2/mir-29c, it has a tumor suppressor effect by targeting MCL-1 and TCL-1 which is an oncogene, and are downregulated in CLL, lung cancer, invasive breast cancer, AML, cholangiocarcinoma, and lung cell cancer lines.[82,83]

Finding targets of animal organisms miRNAs using genome sequences is not as easy as is in plants. While in plants many targets are predicted because of its extreme complementarity84, in animal even though complementarity occurs, is highly unusual.[85] Several target prediction software is available to simplify the look for target genes for miRNAs, nevertheless, lists of possible genes vary according to which program is used86. So, since this approach is not that feasible, there are other approaches to determine targets such as: Up regulation of miRNAs, downregulation of miRNAs, combining

Table 2. Examples of Tumor Suppressor activity of miRNAs

MicroRNA Disease involved Effects
Mir-15a
Mir-16-1
CLL Induces apoptosis and decreases tumor formation.
Let-7 Lung and breast cancers.
Induces apoptosis Mir-29 CLL, AML, Lung and breast cancers, cholangiocarcinoma.
Induces apoptosis and decreases of tumor formation Mir-34 Pancreatic, colon and breast cancers.
Induces apoptosis Genome Scanning candidate's validations

expression, Northern blot analysis, RT-PCR, microarray analysis and RISC purification.

For the up or downregulation of miRNA candidates there are some possible approaches: use of antisense inhibitors, transgenics, specific promoters, and point mutants. Antisense inhibitors (antagomirs or antimirs) blocks the target miRNA function by binding to the mature miRNA and preventing its binding to the to the targeted gene.[87,88] Antagomirs are 2-O- methyloligoribonucleotides and antimirs are[89-91] locked nucleic acid nucleotides containing oligodeoxyribonucleotides. Knocking down the miRNA gene can suppress the miRNA activity and then checking the miRNA profiles to find the specific genes92. Combining overexpression and downregulation of miRNAs is also an approach that has been used in studying mir-14093.

The use of point mutants involves changes in the "seed" sequence, which is of vital importance to identify gene targets, increasing the mismatch of this region by changing one or two nucleotides of it, decreases the gene regulation function of miRNAs.[94] Northern blot analysis is a technique used to study gene expression by detecting RNA or mRNA in a sample.[95] It is reliable enough to study the expression of miRNA in cancers,[49] however it has strong limitations such as unequal hybridization efficiency of individual probes and difficulty in detection of simultaneous miRNAs.[96] Real Time PCR also called quantitative real time polymerase chain reaction (Q-PCR/ qPCR) or kinetic polymerase chain reaction, which is used to amplify and simultaneously quantify a targeted DNA molecule,[97] can be used for quantification miRNA profiles, it's a fast procedure and can detect primiRNA expression, nevertheless it can not correlate between the mRNA expression levels and the up or downregulation of certain miRNAs as the cause of the disease.[98]

DNA microarray is a multiplex technology consisting of an arrayed series of thousands of microscopic spots of DNA oligonucleotides containing picomoles of a specific DNA sequence.[99] It has become a comprehensive technology for comparing normal and tumoral tissues and the miRNA expression between them.[56] Finally, after biochemical purification of RISCs by anti-Ago2 proteins, miRNA targets can be identified since all RISCs are purified by the antibody using microarray hybridization and sequencing.[93,100]

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