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MicroRNAs in Cancer Diagnosis and Treatment

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ABSTRACT

RNA interference is an endogenous process, initially identified as a defense mechanism against invasion of foreign genes (e.g. viruses) in the nematode *Caenorhadbitis elegans*. The non-coding RNA, both long and short, are expressed in organisms and represent a process of posttranscriptional gene regulation, which is used to maintain homeostasis and regulate the expression in almost all living organisms. RNA interference is a recent discovery, but widely used to inhibit gene expression, and is already a valuable research tool to determine metabolic pathways, molecular mechanisms, and pathways of internalization signals that underlie the development of many diseases, including cancer. Cancer is a genetic and epigenetic disease that requires the accumulation of genomic inactivated tumor suppressor genes and activated oncogenes. Recently, a group identified that there are intrinsic suppressor genes and oncogenes with non-coding RNA features that have been called microRNA. These are RNA molecules of 18-24 nucleotides that, when they match the target messenger RNA, regulate the translation. RNA interference technology is a promising gene therapy in various cancers. As well as use in conjunction with chemotherapy or targeted therapies, it may represent a new way to develop resistance mechanisms for inactivation of cancer cells and improve the effectiveness of treatments. (J CANCEROL. 2015;2:64-74)

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INTRODUCTION

A new class of RNA species has been recently identified as an important component in the regulation of gene expression, performing a vital role in the control of normal development in the maintenance of stem cells and in tumorigenesis.

Aside from the already known functions of messenger RNA (mRNA), which is translated to produce proteins, transfer RNAs (tRNA), which act as carriers of amino acids and ribosomal RNA (rRNA), which plays a key role in the ribosome to allow protein synthesis, this new class of small, non-coding RNA known as short interfering RNA or silencing RNA (siRNA) plays a novel regulatory role in gene silencing called RNA interference (RNAi)[1] that focuses on blocking the translation of specific mRNA molecules.

This process was identified in several studies of different species of eukaryotes. Guo and Kemphues (1995)[2] observed that injection of sense and/or antisense strands of RNA selectively suppresses gene expression in the nematode *Caenorhabditis elegans.* Subsequently, Fire, et al.[3] realized that this effect was 10 to 100 times more potent when a mixture of both strands forming double stranded RNA (dsRNA) was injected. As a result, dsRNA was identified as being responsible for the gene silencing caused by RNAi. Following the discovery of this inhibitory pathway, other experiments were conducted in plants, fungi, and fruit flies, which demonstrated the existence of mechanisms of gene silencing mediated by dsRNA[4].

Researchers then wondered whether siRNAs were also encoded in the genome and found the so-called microRNAs (miRNA), which had been discovered years earlier by the Lee group[5]. These miRNAs are RNA molecules that are transcribed from genes, but are not translated into proteins.

It has been postulated that the RNAi mechanism has been evolutionarily conserved in eukaryotes and that it emerged as an early form of innate immunity in cells, with the ability to recognize and silence potentially harmful invading nucleic acids[6], as occurs against viral infections[7]. It has been found in a wide range of organisms, including archaea and eubacteria, reflecting an ancient origin. It has been estimated that there are at least 300 miRNAs (and there may be as many as 1000) in the human genome, targeting the function of between 1-4% of human genes[8].

GENE SILENCING

RNA interference is a process in which siRNAs silence the expression of specific genes by binding to their mRNAs to cause their destruction by blocking their translation or through modifications of chromatin structure. This process begins when siRNAs are endogenously synthesized. In the classical pathway, miRNAs called dsRNAs (double stranded RNA molecules that are 21-24 nucleotides in length with two nucleotide 3' overhangs) are transcribed in the nucleus by RNA polymerase II as long primary transcripts (pri-miRNA) that are processed by Drosha (dsRNA-specific endonucleases) and Pasha (the cofactor for Drosha) enzymes into miRNA precursors that are 70 nucleotides in length.

These precursors are known as pre-miRNA and fold to form imperfect hairpin structures and are then exported into the cytoplasm by an export receptor called exportin-5. Once in the cytoplasm, the Dicer enzyme (another dsRNA-specific endonuclease, an RNA Pol III subtype) generates RNA duplexes that are 21-24 nucleotides in length with two overhanging nucleotides at the 3´ end. For miRNA maturation, the dsRNAs are split into complementary sequences in an ATP-dependent process. The RNA-induced silencing complex (RISC), which incorporates a single strand of miRNA, the Dicer enzyme and a member of the Argonaute (AGO) protein family is subsequently formed, producing an enzyme complex that has endonuclease activity. Based on the miRNA sequence,

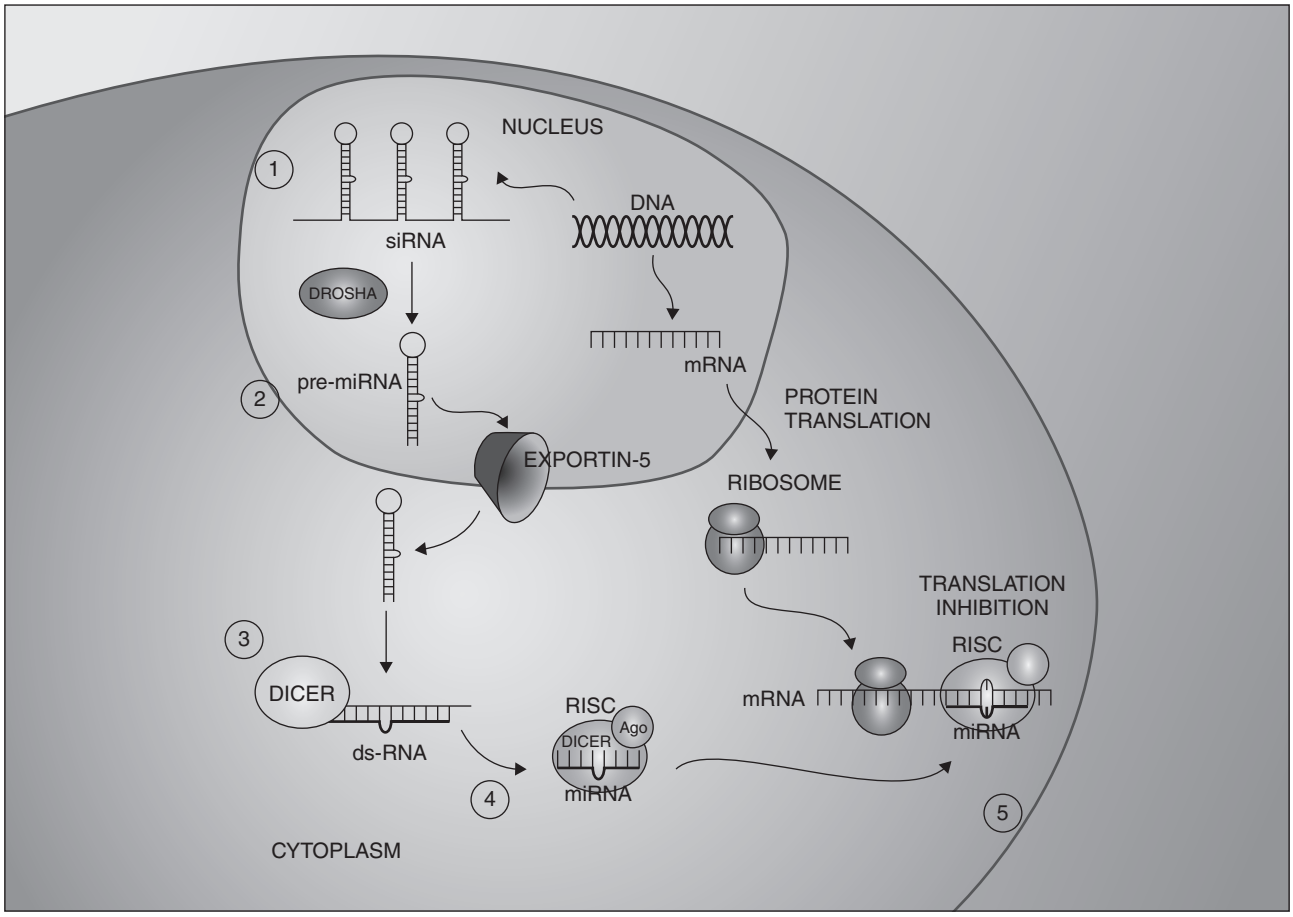

Figure 1. Mechanism of action of microRNAs. The silencing RNAs transcribed in the nucleus by RNA polymerase II (1) are processed by Drosha to generate pre-microRNAs (2). These are translocated into the cytoplasm by exportin-5 (3). Once in the cytoplasm, the enzyme Dicer produces single-stranded RNA 21 to 24 nucleotides long (3). These enter the RNA-induced silencing complex where the single-stranded microRNAs bind to Dicer and Argonaute (Ago2) protein producing an enzyme complex with endonuclease activity (4). Based on the microRNA sequence, RNA-induced silencing complex activates the endonuclease Argonaute 2 that is responsible for cleaving the target messenger RNA of the microRNA in question (5).
miRNA: microRNA; mRNA: messenger RNA; siRNA: silencing RNA; dsRNA: double-stranded RNA; ssRNA: single-stranded RNA; Ago: Argonaute; RISC: RNA-induced silencing complex.

RISC activates the endonuclease activity of Argonaute 2 (AGO2) that is responsible for cleaving the mRNA (Fig. 1).

Silencing can occur in two ways: by binding to mRNA to cause its degradation or by binding to it to block its translation. Which way is used depends on the degree of complementarity between the miRNA and its target at the binding sites within the untranslated regions (UTR) of the respective mRNA.

This in turn depends on: (i) when complementarity is perfect or nearly perfect, the RISC complex is activated and miRNAs bind to their target mRNAs to cause their degradation, and (ii) when the complementarity is not perfect, miRNA binds to its target mRNA and blocks its translation while it is bound to the ribosome.

In animals, inhibition of translation is much more common, as opposed to plants where the degradation of mRNA has been demonstrated[9].

THE ROLE OF MICRORNAS IN CELL DIFFERENTIATION

MicroRNAs exert spatial and temporal control over biological development through the regulation of protein translation. The first study to identify miRNAs was by Victor Ambros[1] who discovered lin-4 RNA, which encodes the "short hairpin RNAs" (shRNA). The inactivation of miRNAs, through the lin-4 and let-7 genes in *C. elegans* epithelial cells, was demonstrated later when it was found that they continued to divide instead of undergoing normal differentiation[10].

It has been shown that expression of miRNAs is required for stem cells to be able to divide continuously. Hatfield, et al. suggest that miRNAs exert inhibition of the Dacapo protein (Dap) at the post-transcriptional level, which in turn inhibits the progression from the G1 phase into the S phase of the cell cycle. Thus, the RNAi pathway allows stem cells to pass through the checkpoint from the G1 to the S phase and to enter the mitosis phase. They suggest that this RNAi-mediated mechanism causes stem cells to be insensitive to environmental signals that would normally stop the cell cycle in the majority of cells[11].

Its importance for development was demonstrated in Dicer knockout mice, where its loss is lethal in early embryonic stages, leading to the depletion of pluripotent stem cells[12].

These studies led to the idea that it is the expression pattern of miRNAs that determines and, therefore, reflects the state of cellular differentiation. Lu, et al. found that the overall expression of miRNAs is different in tumor cells than in their normal counterparts, being significantly higher in the former, especially in those that are more poorly differentiated. At the same time, they analyzed miRNA expression patterns in different tissues and observed that those that shared a common origin, i.e. tissues derived from the same embryonic layer, have similar patterns of expression. This led to the conclusion that the expression patterns of miRNAs not only provide information on the present state, but also retain a memory of the history of the cell differentiation process. In this way, miRNAs make it possible to establish the cell lineage of tissues[13]. On the basis of this evidence, it is speculated that abnormalities in miRNA expression may contribute to the generation and maintenance of tumor stem cells, which have recently been proposed as responsible for cancerous growth in both hematological malignancies as well as solid tumors[14-16].

MICRORNAS AND TUMORIGENESIS

In the past few years, the involvement of miRNAs in tumorigenesis and the process of malignant transformation of normal cells into metastatic cells have been confirmed. The first evidence to confirm this association was reported in a study of B-cell chronic lymphocytic leukemia (B-CLL). Calin, et al. observed in B-CLL specimens that the expression of the genes from which miRNAs miR-15 and miR-16 originate, in chromosome region 13q14 (65% of cases with B-CLL present deletion of this region), is down-regulated or deleted in patients with B-CLL[17].

With regard to solid tumors, there is evidence that miRNA Let-7 has tumor-suppressor activity, given that its expression in lung adenocarcinoma cell lines inhibits colony formation. Likewise, loss of Let-7 is correlated with increased expression of the RAS protein in lung tumors, thereby showing one of the mechanisms through which Let-7 may contribute to carcinogenesis.

There are regulatory and effector proteins, components of the miRNA machinery involved in tumorigenesis. The Dicer protein has been examined by Karube, et al., who found that reduced expression of this protein correlates with lower postoperative survival in patients with lung cancer[18].

Drosha, DGCR8 and Dicer are the best-known regulators of miRNA processing; defects in their

Table 1. Tumor survival mechanisms related to microRNAs

Vital tumor mechanisms	microRNA
Evasion of apoptosis	miR-221, miR-222, miR-15, miR-16, miR-34, miR-21
Unlimited replicative potential	miR-34, miR-373, miR-24
Sustained angiogenesis	miR-17/92, miR-126, miR-424, miR-101
Tissue invasion and metastasis	miR-200, miR103/107, miR-10B, miR-9, miR-31
Self-replication	Let-7, miR-21, miR 17/92

mechanisms are also related to oncogenesis. This has been confirmed by several studies. One of the best known was performed by Merritt, et al., who found that overexpression of Dicer and Drosha mRNA in patients with ovarian, breast, and lung cancer was associated with improvements in overall and disease-free survival (Merritt, et al., 2008)[19].

A few years ago, the interaction of protein p53 with the Drosha microprocessor complex, which facilitates the processing of pri-miRNAs into pre-miRNAs, was shown[20]. The association of protein p53 with the process of miRNA biogenesis is the likely cause of the relationship between downregulation of many miRNAs in which p53 is dysfunctional[13].

It is said that other proteins are also involved in the initial stages of cancer development and AGO1 (also known as EIF2C1), EIF2C2, and Hiwi proteins, all members of the Argonaute family of proteins, have been identified as having a role in the tumorigenesis in tissues, showing a delay in cell differentiation[21]. In Wilms' tumor, overexpression of AGO1 correlates with loss of tumor suppressor activity of the WT1 gene[21]. The Hiwi protein, whose gene is located on chromosome 12 (an ortholog of the Argonaute-Piwi gene family in Drosophila melanogaster), has been linked to the development of testicular germ cell tumors, showing altered activity levels as well as an increase in its expression in seminomas[22].

Michael, et al. studied the presence of miRNAs in human colonic adenocarcinoma and identified 28 different miRNA sequences, three of which were previously unknown and seven that had only been cloned in rats. A significant and steady reduction in the mature miRNA levels of the miR-143 and miR-145 genes from early adenomatous to very advanced stages of colorectal neoplasia was found[23].

Calin, et al.[24] performed a study to analyze the sites of several miRNA genes in the genome to investigate whether they were located in regions associated with cancer. These regions included sites that are known for the amplification, deletion and loss of heterozygocity frequently found in several types of cancer as well as fragile sites in the genome. The results confirmed that a great many miRNA genes are located in genomic regions associated with cancer, and a thorough examination of these regions showed that they contain other miRNAs that have not yet been identified. Surprisingly, more than 50% of currently known miRNAs are located at or near fragile sites or in minimal regions of loss of heterozygocity, minimal regions of amplification and common breakpoint regions associated with cancer[24,25] (Table 1).

MICRORNAS AS ONCOGENES (ONCOMIRS) AND AS TUMOR SUPPRESSOR GENES (MIRSUPPS)

There is a hypothesis regarding siRNA-based regulatory mechanisms, i.e., that they may interfere in tumorigenesis through oncogene and tumor suppressor gene functions. MicroRNAs with tumor suppressor function (also known as mirsupps) may be considered to be those that cause downregulation of cancer and target oncogenes, whereas

oncogenes (or oncomirs) are upregulated miRNAs that target tumor suppressor genes or act as differentiation genes[26]. They may also participate in a post-transcriptional collapse, in which the altered expression of the miRNA causes a post-transcriptional misregulation of an oncogene or tumor suppressor gene. It is believed that their function depends on their location in the genome and the specific chromosomal abnormality of the type of cell that is affected.

MiR-155 was the first miRNA transcript to show oncogenic activity and has been identified as essential for the development of the immune system. It is upregulated in classical Hodgkin lymphoma, diffuse large cell lymphomas, and breast and lung cancers.

MicroRNAs may also be organized into clusters, the best-known and studied to date is a complex or cluster of six miRNA called miR-17-92.

Arabi, et al.[27] showed that transcription of the miR-17-92 cluster is activated by MYC, which in turn mediates cell proliferation. Another target of miR-17-92 is E2F1, which is a transcription factor that promotes progression from G1 to S phase. This cluster has both tumor-suppressor gene and oncogene features. As an oncomir, it acts by negatively regulating E2F1 apoptotic activity.

Oncogenic activation of the RAS gene induces cellular senescence and if this is combined with loss of p53, the cells are able to overcome this response and continue to proliferate. In one study, while screening for miRNAs that cooperate with oncogenes to permit cellular transformation, Voorhoeve, et al.[28] used a library of vectors expressing miRNAs to identify miR-372 and miR-373 as inducers of proliferation and tumorigenesis of primary human cells in cooperation with RAS by also rendering cells insensitive to the effects of a normally functioning p53 allele through direct inhibition of the expression of the gene known as LATS2 (large tumor suppressor, homolog 2). LATS2 is a known inhibitor of CDK2 and induces cell cycle arrest. They found that oncogenic growth is enabled due to the participation of this mechanism in the oncogenesis of testicular germ cell tumors as it affects the endogenous p53 pathway[28].

Another gene, classified as an oncogene, that has grown in importance is miR-21. This miRNA is overexpressed in glioblastoma, pancreas, and breast cancer. It was shown that aberrant expression of miR-21 promotes tumorigenesis through the inhibition of the apoptosis pathway, and that by the silencing of the latter by means of anti-miR-21 (an oligonucleotide used to silence miRNAs), it is responsible for inhibiting tumor growth. In an analysis of 157 miRNAs using reverse transcription polymerase chain reaction (RT-PCR), miR-21 was the most abundantly expressed in breast tumor tissue[29]. Using proteomic analysis, the tumor suppressor tropomyosin-1 (TPM1) was identified as a potential target of miR-21. And its expression is absent in epithelial cells derived from breast cancer tissue.

Tumor suppressor genes, as has been mentioned, act differently, and it was a study of leukemia published by Calin, et al. that was the first to identify that miRNAs miR-15a and miR-16-1 may function as tumor suppressor genes. In a more recent study it was shown that miR-15a and miR-16-1 negatively regulate BCL2, which is an anti-apoptotic gene[30].

There are other miRNAs in this group, among the most prominent of which are miRNA-143 and 145, whose expression levels are reduced in colon cancer cells.

The reduced expression of the let-7 miRNA has been documented in several tumor types and its function as a tumor suppressor is attributed to its ability to inhibit the RAS oncogene[31], like MYC and HMGA2. This miRNA is poorly expressed primarily in colon and lung tumors and their cell lines.

Since the first reports that were published on the oncogenic mechanisms of miRNAs, their targets, the mechanisms involved and their involvement as

Table 2. Studies to date of microRNAs in solid tumors that present with clinical applications

Type	microRNA	Cancer	Function	Clinical application
Oncomirs	*miR-155*	Breast	Microsatellite instability increases. Positive regulation by NF-κB.	Prognosis
	miR-17/92 family	Lymphoma, lung, pancreas, liver cancer	Regulated by MYC. Modulates E2F1 thus regulating cell proliferation and apoptosis.	Prognosis
	miR-21	Lung Breast, lung, pancreas, prostate	Increases tumorigenesis by stimulating K-RAS. Promotes cell proliferation and metastasis. Upregulation by IL-6 and GF1α.	Possible therapeutic target, prognostic factor of response to chemotherapy
Mirsuppr	*Let-7*	Lung, ovary	Inhibits RAS.	Prognostic and predictive factor of chemoresistance
	miR-145	Breast, colon	Reduces cell proliferation and induces apoptosis.	Prognosis
	miR-143	Colon	Inhibits cell growth.	
	miR-122	Liver cancer	Reduces cell proliferation, metastasis and cell survival.	Prognosis
	miR-34	Colon, lung	Acts on the p53-miR-34 circuit, inhibiting proliferation and cell survival. Regulates methylation.	Diagnosis and prognosis

NF-κB: nuclear factor kappa B; IL: interleukin; K-RAS: Kirsten rat sarcoma; GF1α-: growth factor 1 alpha; miRNA: microRNA.

oncogenes or tumor suppressor genes have continued to be studied to evaluate their potential role as biomarkers for diagnosis, prognosis and treatment[32] (Table 2).

THE ROLE OF MICRORNAS IN VIRAL INFECTIONS ASSOCIATED WITH CANCER

Certain viral infections, such as the human papillomavirus (HPV) in cervical cancer, Epstein-Barr virus (EBV) in Burkitt's lymphoma, the hepatitis B and C viruses (HCV and HBV) in hepatocellular carcinoma and herpes simplex virus 8 (HVS8) in Kaposi's sarcoma, among others, have been associated with the development of cancer.

Although the mechanisms of virus-induced tumorigenesis are entirely unknown, entry of the viral genome into the cell is considered essential for its malignant transformation.

Cellular antiviral defense mechanisms are another form of cell-virus interaction. As mentioned above, miRNA are considered to be highly conserved mechanisms that have among their primary functions to act as a defense against invading nucleic acids that can be potentially damaging, such as viruses. It is known that certain oncogenic viruses integrate their DNA into the host cells. A classic example is to be found in cancer of the cervix, in which the presence of high-risk HPV types has been identified in virtually 100% of tumor tissue[33]. When HPV is integrated into the host cell genome, it expresses two oncogenes, E6 and E7, which inhibit the functions of proteins p53 and retinoblastoma (pRB) respectively. This enables an increased rate of cell division that promotes genetic instability, which predisposes for malignant transformation. It has been observed that the insertion of siRNA directed against the mRNA of E6 and E7 in neoplastic cells decreases their levels and leads to the reactivation of the p53 protein. Studies on this subject have produced controversial results as some have shown that the activation of this protein inhibits cell growth without significant induction of apoptosis[34,35] whereas others have shown massive apoptosis in cancerous cervical cells following insertion of siRNAs (Butz, et al., 2003)[36].

However, viruses have also evolved and developed in turn mechanisms to evade this gene silencing[37]. It is now known that viruses also express miRNAs (v-miRNAs), which may act as self-regulators. It has been observed that these v-miRNAs can interfere with the host cell transcripts (Cullen, et al., 2006[38]). The ebv-miR-BART5 in Epstein-Barr virus falls into this category, being responsible for the regulation the p53 protein and a modulator of apoptosis called PUMA in infected cells; this makes the cells less sensitive to pro-apoptotic agents, promoting cell survival[39].

In recent reports computational predictions have been made using current databases, where it has been seen that p53, cyclin G2 and other genes that regulate apoptosis are targets for a high percentage of v-miRNAs, which act as oncogenes or directly promote oncogenic development by the use of external mechanisms[40].

MICRORNA EXPRESSION PROFILES IN THE DIAGNOSIS AND PROGNOSIS OF CANCER

The use of miRNA microarrays has allowed expression studies of multiple genes, confirming that miRNAs are expressed differently in normal and tumor tissues. These profiles have also proven to be an invaluable source of information, by reflecting the developmental lineage and differentiation state of different tumors. With the wealth of information that the expression pattern of miRNAs provides on the lineage and differentiation state of a cell, it has been possible to classify very poorly differentiated tumors by using miRNA expression profiling methods. Owing to their higher specificity, miRNAs have been shown to be superior to mRNA profiles in the successful classification of tumor tissues. These findings raise the question about the important role that miRNA profiles may have in cancer diagnosis, especially in poorly differentiated tumors[13].

By studying tumor cells with a common developmental lineage, it was observed that their miRNA expression patterns differed. A library of miRNA signatures or miRNA expression profiles, determined according to the specific tumor types, could help both in the diagnosis and the prognosis as well as predictive biomarkers of response to treatment (Fig. 2). A classic example came from a study where patients with lung cancer were classified into two groups according to let-7 expression and which showed that cases with reduced let-7 expression presented with shorter survival following surgical resection[13]. In a study of patients with pancreatic ductal adenocarcinoma who were treated with gemcitabine, it was found that high expression of miR-221 was associated with a significant decrease in survival in both metastatic disease and during adjuvant therapy (Giovannetti, et al., 2010)[41].

Another way in which miRNAs can be used for diagnostic purposes is in the differentiation of tumor tissue from normal tissue, or in cancers caused by chronic inflammation. This use was studied by Bloomstom, et al. (Bloomstom, et al., 2010)[42], who used microarrays to identify miRNAs that were overexpressed and underexpressed in tissue from patients with pancreatic cancer or chronic pancreatitis and compared them with normal pancreatic tissue, finding these were correctly differentiated with 90% and 93% accuracy respectively.

A subject that is very important but to date has been little studied is the distinction between primary and metastatic tumors as well as between tumors with low and high metastatic potential. The elevated expression of miR-103/107 is associated with high probability of metastasis and poor outcomes. It is noted that miR-103/107 increases migration, invasion and metastasis through its functional target, the DICER protein. Furthermore, this miRNA in turn controls the mesenchymal-epithelial transition, leading to cell motility, invasion and hematogenous dissemination; it does this through a family called miR-200[43].

MiRNA expression has also been associated with the drug resistance of tumor cells[44], turning them into pharmacogenomic markers.

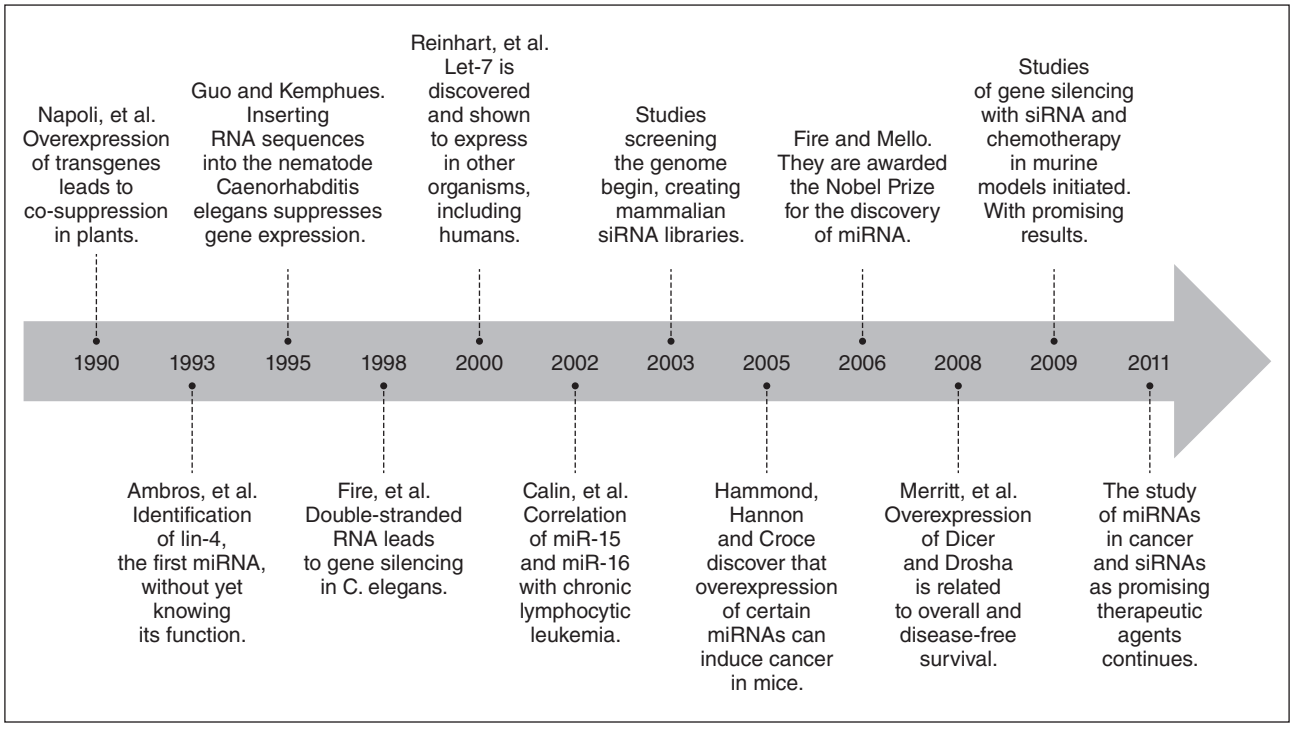

Figure 2. MicroRNAs and Cancer. The timeline of events related to microRNAs and their involvement in cancer is shown. miRNA: microRNA; siRNA: silencing RNA.

MICRORNAS IN CANCER TREATMENT

The process of carcinogenesis in humans involves a number of steps requiring between 7-10 discrete genetic and epigenetic events that persist and whose continuous production is necessary for maintenance of tumor viability[45]. Oncogenes found in cancer differ from their normal counterparts by single base mutations only. Technology to specifically inactivate the mutant versions of these oncogenes may be essential for effective antitumor therapy. MicroRNAs may be able to participate in this selective silencing. Brummelkamp, et al.[46] delivered siRNAs through a viral vector to specifically inhibit mutated K-RASV12 allele in human pancreatic carcinoma, without modifying the wild-type K-RAS allele.

However, there are studies that indicate that exclusive use of RNAi would not be sufficient to kill human tumor cells, although it could be used as adjuvant treatment with chemotherapy in certain tumors. Koivusalo, et al.[47] studied this possibility in a study that transfected siRNAs targeted against the mRNA of the E6 and E7 genes of HPV18 in cervical cancer cells and followed with chemotherapy on the hypothesis that there would be a greater response to treatment. They observed that expression of p53 increased only transiently in spite of the continuous suppression of the oncogene mRNA levels mediated by siRNAs and that it did not result in the induction of apoptosis in the tumor cells. However, when chemotherapy was added, there was a steady increase in p53 activity in the neoplastic cells, resulting in effective inhibition of tumor growth.

Therapeutic strategy is based both on introducing synthetic RNAi into cells to block specific genes, together with locating a target miRNA and blocking its action. For the latter, there are synthetic antisense oligonucleotides that encode sequences that are complementary to the mature oncogenic miRNAs, called anti-miRNA oligonucleotides (AMO), in the

hope that they might effectively inactivate miRNAs that contribute to tumorigenesis or allow their growth to be slowed. At present. phase 0 studies and trials are being conducted, in which a special form of AMOs conjugated with cholesterol, called antagomirs, which have shown inhibitory activity in various organs when injected into mice, is administered[48]. From studies conducted to date, it is hypothesized that miRNAs have therapeutic potential as an adjuvant to chemotherapy without being the "magic bullet" that they were thought to be at one point, but very useful when they are used in combination with drug therapy.

PERSPECTIVES

At present *in vivo* experiments continue to be conducted in search of better methods for the transfection of miRNAs, to make them more specific and enable many pathways to be blocked, without losing organ specificity. Studies of blocking mechanisms of action and knockout organisms will allow an understanding of the pathways that would be affected by the manipulation of miRNAs. Together with gene expression profile studies and proteomic analysis, association studies with gain- or loss-of-function studies of miRNAs in both *in vivo* and *in vitro* models will help improve understanding of the molecular pathways controlled by these RNAs in specific tumors and thus to identify potential therapeutic targets.

Preclinical studies have demonstrated a lack of toxicity for both siRNAs and antago-mirs in *in vivo* models, so there is excitement and plans to conduct phase I studies of this new therapeutic tool, focusing on routes of administration and tissue specificity to avoid adverse effects and to determine the pharmacokinetics of these molecules.

Nevertheless, we should not overlook the fact that owing to differences in the approach to its uses and mechanisms of action, miRNA modulation is a new kind of therapy still under investigation.

CONCLUSION

RNAi in its various forms constitutes a new class of gene regulatory mechanisms as well as a therapeutic tool, whose role in the genesis, progression, and treatment of cancer is only beginning to be understood. In the coming years we will see how this mechanism is incorporated into diagnostic tools for screening and for use in the treatment of some cancers, thus leading to a more precise and advanced understanding of the disease.

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