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miRNAs in Human Cancer

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Abstract

MicroRNAs (miRNAs) are small (~18–25 nucleotides), endogenous, noncoding RNAs that regulate gene expression in a sequence-specific manner via the degradation of target mRNAs or the inhibition of protein translation. miRNAs are predicted to target up to one-third of all human mRNAs. Each miRNA can target hundreds of transcripts and proteins directly or indirectly, and more than one miRNA can converge on a single target transcript; thus, the potential regulatory circuitry afforded by miRNAs is enormous. Increasing evidence is revealing that the expression of miRNAs is deregulated in cancer. High-throughput miRNA quantification technologies provide powerful tools to study global miRNA profiles. It has become progressively more apparent that, although the number of miRNAs (~1,000) is much smaller than the number of protein-coding genes (~22,000), miRNA expression signatures more accurately reflect the developmental lineage and tissue origin of human cancers. Large-scale studies in human cancer have further demonstrated that miRNA expression signatures are associated not only with specific tumor subtypes but also with clinical outcomes.

Keywords

MicroRNA; Noncoding RNA; Cancer

1. Introduction

Cancer is a disease involving multistep changes in the genome (1). Recent studies have focused mainly on protein-coding genes, and little is known about the alterations of functional noncoding sequences in cancer (2–4). MicroRNAs (miRNAs) are small (~18–25 nucleotide), endogenous, noncoding RNAs that regulate gene expression in a sequence-specific manner (5–8). Rapidly accumulating evidence indicates that miRNAs are involved in the initiation and progression of cancer in several ways. First, miRNAs act as key regulators of various fundamental biological processes that share common pathways with cancer, such as development, differentiation, apoptosis, and cell proliferation (5–7). Second, increasing evidence shows that the expression of miRNAs is markedly deregulated in cancer due to multiple genomic and epigenetic alterations (2, 9–22), and third, several miRNAs have been shown to serve as tumor suppressor genes or oncogenes (3, 12, 19, 20, 23–34). The investigation of miRNAs in cancer may provide novel strategies for both the diagnosis and treatment of this disease.

2. Biogenesis of miRNAs

With the exception of those from the Alu repeat regions, which are transcribed by RNA polymerase III (Pol III) (35), most miRNA genes are derived from primary miRNA

transcripts (pri-miRNAs) which are produced by Pol II and contain a 5' cap and a poly(A) tail (36, 37). The pri-miRNA is cleaved within the nucleus by a multiprotein complex called Microprocessor, which is composed of the RNase III enzyme Drosha and the double-stranded RNA-binding domain (dsRBD) protein DGCR8/Pasha (38–42), into a ~70-nt hairpin precursor known as pre-miRNA. Next, the pre-miRNA is exported into the cytoplasm by Exportin-5 via a Ran-GTP-dependent mechanism (43–45). The pre-miRNA is further cleaved into a mature ~22-nt miRNA:miRNA* duplex by an RNase III enzyme, Dicer, in association with its partners, TRBP/Loquacious and PACT in human cells (46, 47). Subsequently, an RNA-induced silencing complex called RISC is assembled with the protein Argonaute (Ago) 2 (48, 49). The miRNA strand is selectively incorporated into the RISC complex (50, 51) and guides the complex specifically to its mRNA targets through base-pairing interactions (Fig. 1).

3. miRNA Silencing Mechanisms

miRNAs can downregulate the expression of their target genes via two different mechanisms; the mechanism used depends on the complementarity between the miRNA and its target. miRNAs with perfect or near-perfect complementarity to the target sequence induce the cleavage and degradation of the transcript by initiating deadenylation and decapping of the mRNA (52). However, most miRNAs bind imperfectly to their target sequences and function by repressing protein translation. The underlying molecular mechanisms resulting in this repression have been studied intensively using in vitro cell-free systems (53). Recently, Wakiyama et al. established a cell-free system derived from human embryonic kidney (HEK) 293 cells and demonstrated that efficient miRNA-guided translational repression requires a m⁷G-cap as well as a poly(A) tail (54); this is consistent with a previous report using a rabbit reticulocyte lysate system (55). In addition, a study utilizing extracts from mouse Krebs-2 ascites cells (56) showed that inhibition of translation initiation can be due to changes in ribosome recruitment to the mRNA as well as targeting of the mRNA cap structure. The dilemma of how the miRNA ribonucleoprotein complex (miRNPs) that is bound to the 3' UTR of a target mRNA interferes with the initiation of translation was resolved by Kiriakidou et al. (57). They identified a motif (MC) within the Mid domain of Ago proteins, which bears significant similarity to the m⁷G cap-binding domain of eIF4E, an essential translation initiation factor. In their model, the Ago proteins compete with the eIF4E for cap binding and thus repress the initiation of translation. Inhibition of translation initiation by *mir-2* was similarly observed in a cell-free system from *Drosophila* embryos. Interestingly, *mir-2* induced the formation of structures (heavier than 80S) known as “pseudo-polysomes,” which resemble cytoplasmic processing bodies (P bodies) (58).

4. Oncomirs in Cancer

Changes in miRNA expression and function may contribute to the initiation and maintenance of tumors. Such miRNAs have been referred to as “oncomirs,” and they may serve as both tumor suppressors and oncogenes (3). The first indication of miRNAs as tumor suppressors came from a report by Calin et al. where they found that patients diagnosed with B-cell chronic lymphocytic leukemia (B-CLL) have frequent deletions or downregulation of

the *mir-15a* and *mir-16-1* genes on chromosome 13q14.3 (23). A follow-up study demonstrated that *mir-15a* and *mir-16-1* negatively regulate the antiapoptotic protein BCL2 at a posttranscriptional level (59), and their function in leukaemogenesis and lymphomagenesis has been supported by additional studies (9, 10, 60). Some other miRNAs have also been shown to function as tumor suppressor genes, for example, the *let-7* family, which are negative regulators of RAS (26). Other miRNAs can function as oncogenes. The *mir-17-92* cluster was found by He et al. (25) to be upregulated in 65% of B-cell lymphomas, and its overexpression accelerated the development of malignant lymphomas in a transplantation mouse model. Upregulation of *mir-21* has been reported in glioblastomas (24) and breast cancer (61), where it exerts an antiapoptotic function. *mir-155* is remarkably overexpressed and linked to tumorigenesis (likely in cooperation with MYC) in pediatric Burkitt, Hodgkin, primary mediastinal, and diffuse large-B-cell lymphomas (62–65) as well as in breast cancer (61). In addition, *mir-372* and *mir-373* have been implicated as oncogenes in testicular germ cell tumors (28).

5. Deregulation of miRNAs in Cancer

The underlying mechanisms of miRNA deregulation in human cancer are not well understood, however, recent findings indicate that multiple processes are involved (21). It has been well documented that most primary miRNAs are transcribed from Pol II promoters that are regulated by transcription factors (5–8, 36, 37), and several examples of miRNA deregulation in cancer due to transcriptional deregulation have been reported (27, 29, 66–68). Recent studies also suggest that epigenetic alterations play a critical role in deregulating miRNA expression in human cancers (12, 21, 69), and that mutations may contribute to the downregulation of mature miRNAs (10). Over 50% of miRNAs are aligned to genomic fragile sites or regions associated with cancers (13), and several groups have provided evidence that DNA copy number abnormalities are involved in miRNA deregulation (14, 23, 25). Finally, the key proteins in the miRNA biogenesis pathway may be dysfunctional (70) or deregulated in cancer (71–74), thereby enhancing tumorigenesis (75). Thus, transcriptional deregulation, epigenetic alterations, mutations, DNA copy number abnormalities, and defects in the miRNA biogenesis machinery might contribute either alone, but more likely together, to the deregulation of miRNAs in human cancer.

Global expression of miRNAs is seemingly deregulated in most cancer types, according to reports from recent high-throughput studies (2, 9–11, 21, 76, 77). Interestingly, some studies suggest that miRNA expression may be widely downregulated in human tumors relative to normal tissues, as revealed by bead-based flow cytometry (9) and miRNA microarrays (21). However, other microarray studies have reported a tumor-specific pattern of down- and upregulation of miRNA genes (10, 11, 76). The selection of control samples may therefore be critical in the interpretation of these results. For example, normal ovaries are composed mainly of stroma, with smaller amounts of surface epithelium, thus the whole ovary may not serve as an optimal control for epithelial ovarian cancer studies (78). Recently, miRNA profiles in ovarian cancer were reported by several independent groups, using different control samples (17, 20, 21, 79). Although the overall conclusion of these studies is consistent, i.e., the expression of miRNAs is highly deregulated in ovarian cancer, the

detailed expression patterns of each study are distinct, highlighting the challenge of determining an appropriate control tissue sample for profiling studies.

Transcriptional regulation is one of the key steps controlling the expression of miRNA, and several studies have demonstrated that miRNA deregulation in cancer can be due to changes in miRNA transcription. The expression of several miRNAs, including the oncogenic miRNA *mir-17-92* cluster, is regulated by the transcriptional factor c-Myc (27, 66). It has been shown that *c-Myc* is amplified and overexpressed in several types of human tumors, which suggests that this may contribute to the upregulation of *mir-17-92* in cancer (27, 66). In addition, the miRNA tumor suppressor *mir-34* is regulated by the transcription factor p53 (29, 80–84), and p53 inactivation is believed to decrease *mir-34* expression in human cancers (85). Finally, activation of the transcriptional factor HIF may be important to the upregulation of *mir-210* expression in human cancer (67, 68, 86). Taken together, an increasing amount of evidence indicates that transcriptional deregulation plays an important role in the deregulation of miRNAs in human cancer.

Epigenetic changes such as DNA methylation and histone modification play an important role in chromatin remodeling and in the general regulation of protein-coding gene expression in human cancer (87). Likewise, such mechanisms may also function to affect miRNA expression in cancer. To test this hypothesis, several groups treated cancer cell lines with DNA-demethylating reagents and/or histone deacetylase inhibitors in vitro and monitored miRNA expression by microarray analysis (12, 76, 88, 89). The results suggest that epigenetic alterations may play a critical role in regulating miRNA expression in human cancers, and therefore epigenetic treatments may provide novel strategies for cancer therapy.

Alterations in DNA copy number is one mechanism that can modify gene expression and function, and DNA dosage alterations in somatic cells are frequent contributors to cancer (90). The first example of a miRNA gene with an alteration in DNA copy number in cancer was reported in CLL patients. The genes *mir-16-1* and *mir-15a* on chromosome 13q14 were deleted in more than 50% of the CLL patients studied, with concurrent reduced expression in ~65% patients (23). Additional studies demonstrated that these two miRNAs suppress *BCL2* expression and may serve as tumor suppressor genes in this disease (59). Deletions of *mir-16-1* and *mir-15a* were later identified in epithelial tumors, such as pituitary adenomas (91), and in ovarian and breast cancers (14). In 2004, the amplification of *CL3orf25* on chromosome 13q31-32 was first reported in lymphoma patients (92). Interestingly, this amplified region contains seven miRNAs as a polycistronic cluster, and there was an increased expression of primary and mature miRNAs derived from this locus in this type of lymphoma (25, 93). We now know that this miRNA cluster actually serves as an oncogene in human cancer (25, 94, 95) by altering the balance between cell death and proliferation via a *c-Myc* mediated pathway (27, 95). Using a bioinformatics based approach on data obtained from public databases, Calin et al. (13) compared 186 miRNA loci to the sequences of previously reported nonrandom genetic alterations and found that miRNA genes frequently reside in fragile sites, as well as in minimal regions of loss of heterozygosity, minimal regions of amplification, and common breakpoint regions. Recently, this finding was experimentally confirmed in an array-based comparative genomic hybridization (aCGH) study in 227 human tumors (14). A more recent study has suggested that a loss in genomic

copy number may account for the downregulation of approximately 15% of miRNAs in advanced ovarian tumors (21). These findings support the notion that alterations in the DNA copy number of miRNA genes are highly prevalent in cancer and may account in part for miRNA gene deregulation.

6. miRNA-Based Cancer Therapy

The binding of miRNAs to their targets are governed by the rules of Watson–Crick base pairing. Therefore, an obvious molecule that could be used to inhibit an miRNA is an anti-miRNA oligonucleotide (AMO), which competitively blocks the interaction between the miRNA and its target (96). AMOs can be chemically modified in a variety of ways to improve their stability. One example is a locked nucleic acid (LNA), often referred to as inaccessible RNAs, which is a bicyclic high-affinity RNA analogue where the ribose moiety is chemically locked in an RNA-mimicking N-type (C3'-endo) conformation by the introduction of an extra 2'-O, 4'-C methylene bridge (97). The locked ribose conformation enhances base stacking and backbone pre-organization and significantly increases the thermal stability upon hybridization with complementary single-stranded RNA target molecules. In addition, LNAs are compatible with RNase H cleavage and display high aqueous solubility and low toxicity in vivo (98). Other oligonucleotide analogues, such as morpholinos (99), 2'-O-methyl-(100), and 2'-O-methoxyethyl-modified (2'-MOE) oligonucleotides (101) have also been shown to be efficient in functionally inhibiting miRNAs. Besides chemical modifications, some improvements in inhibitor potency have been observed by increasing the length of the AMOs (102). Optimized secondary structural elements that flank the antisense core have also been shown to be highly potent and specifically block RISC activity in vitro for extended periods of time, thus suggesting structures surrounding or adjacent to the antisense core sequence are major determinants of inhibitor potency (103). In summary, a combination of optimization of sequences, structures, and/or chemical modifications may be required to produce a potent AMO.

Since protein-coding tumor suppressor genes can inhibit tumor growth, it has been proposed that restoring tumor suppressive miRNAs may also have an antitumorigenic effect. An example of miRNA replacement therapy is with *mir-15* and *mir-16*, which target BCL2 (10) and are often deleted in CLL patients (23). It has been reported that the transfection of *mir-15/16* expressing constructs resulted in the reduction of BCL2 protein levels and increased apoptosis in cancer cell lines. This study highlights the possibility of treating tumors displaying BCL2 overexpression by restoring *mir-15a* and *mir-16-1* expression. Another therapeutic candidate is *mir-124a*, whose expression is downregulated in acute lymphoblastic leukemia (ALL) due to hypermethylation of the promoter as well as histone modifications, resulting in an upregulation of the expression of target genes, including CDK6, and the phosphorylation of retinoblastoma (Rb). Accordingly, forced expression of *pre-mir-124a* led to decreased tumorigenicity in a xenogeneic mouse model of ALL (104). Mendell et al. have recently demonstrated that *mir-26a* in hepatocellular carcinoma (HCC) represents an additional example of a tumor suppressing miRNA, and that systemic administration of this miRNA using adeno-associated virus (AAV) in an animal model of HCC results in inhibition of cancer cell proliferation, induction of tumor-specific apoptosis, and significant protection from disease progression without toxicity (105).

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Fig. 1.

Biogenesis of miRNAs. miRNA genes are derived from primary miRNA transcripts which are produced by Pol II and contain a 5' cap and a poly(A) tail. The pri-miRNA is cleaved within the nucleus by a multiprotein complex called Microprocessor into a ~70-nt hairpin precursor known as pre-miRNA. Next, the pre-miRNA is exported into the cytoplasm by Exportin-5. The pre-miRNA is further cleaved into a mature ~22-nt miRNA:miRNA* duplex by an RNase III enzyme, Dicer. Subsequently, an RNA-induced silencing complex called RISC is assembled with the protein Argonaute (Ago) 2. The miRNA strand is selectively incorporated into the RISC complex and guides the complex specifically to its mRNA targets through base-pairing interactions.