MicroRNAs in human cancer: from research to therapy

Massimo Negrini[1,*], Manuela Ferracin[1], Silvia Sabbioni[1] and Carlo M. Croce[1,2]

[1]Dipartimento di Medicina Sperimentale e Diagnostica, Centro Interdipartimentale per la Ricerca sul Cancro, Università di Ferrara, via Luigi Borsari 46, 44100 Ferrara, Italy

[2]Comprehensive Cancer Center, Ohio State University, Columbus OH 43210, USA

*Author for correspondence (e-mail: ngm@unife.it)

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Summary

Numerous miRNAs are deregulated in human cancers, and experimental evidence indicates that they can play roles as oncogenes or tumor suppressor genes. Similarly to cancer genes that encode proteins, deregulation of miRNA-encoding genes is associated with genetic or epigenetic alterations, such as deletions, amplifications, point mutations and aberrant DNA methylation. The discovery that miRNAs interact with known oncogenes has established further links with molecular pathways implicated in malignant transformation. Finally, miRNAs can be used as diagnostic markers, and their potential as therapeutic molecules has moved miRNAs from the area of basic research to the field of cancer biotechnology.

Key words: microRNA, Cancer, Microarray, Oncogenes, Tumor suppressor genes

Introduction

Malignant transformation is a process that involves genetic and epigenetic alterations that influence cell growth, death and differentiation. At later stages in tumorigenesis, additional changes may promote angiogenesis, invasion of surrounding tissues and metastasis to distant sites. With few exceptions, this multifactorial and multistep process proceeds over numerous years. The genes involved in cancer are generally mutant forms of normal genes that have become either activated or inactivated and are known as oncogenes or tumor suppressor genes, respectively. Understanding the biological functions of these genes has led to the identification of molecular pathways and networks whose deregulation is responsible for cancer initiation and progression.

Most known cancer genes encode proteins, and our ability to recognize their association with human cancer was made possible by the identification of genetic and epigenetic changes that affect the functions of their protein products. Hence, many techniques have been developed over the years for investigating protein-coding genes and their potential involvement in human cancer. However, in recent years a class of genes that encode tiny RNAs called microRNAs (miRNAs) have been found to be altered in human cancer. Despite our still limited arsenal of techniques for studying the functions of genes encoding miRNAs, several lines of evidence indicate that miRNAs play an important role in malignant transformation of human cells.

The miRNAs lin-4 and let-7 were the first to be discovered and shown to function in *Caenorhabditis elegans* as triggers for a cascade of gene expression that regulates developmental events by post-transcriptional gene silencing (PTGS) (Lee et al., 1993; Reinhart et al., 2000; Wightman et al., 1993). Initially believed to play a regulatory role only in worms, their importance became more apparent in 2001 when miRNAs were identified and cloned from several organisms, including human, and their nucleotide sequences were found to be philogenetically conserved (Pasquinelli et al., 2000; Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001).

The most recent release of the miRBase Registry (http://microrna.sanger.ac.uk/) (Griffiths-Jones et al., 2006) (9.1, released on February 2007) lists >4000 different miRNAs identified in animals, plants and viruses, and 474 human miRNAs. Computational algorithms predict that as many as 1000 miRNAs may exist in the human genome (Berezikov et al., 2005).[†]

Excellent reviews describing the molecular biology of miRNAs have been published elsewhere (Bartel, 2004; He and Hannon, 2004). Here, we just summarize a few essential elements. In humans, miRNA genes are located in all chromosomes, with the exception of the Y chromosome. Nearly 50% of known miRNAs are found in clusters and transcribed as polycistronic transcripts. The majority of mammalian miRNA genes are located in introns of protein-coding genes or in intergenic non-protein-coding transcriptional units; less often they reside in exons, displaying an anti-sense orientation with respect to the protein-coding gene (for a review, see Kim and Nam, 2006). Intergenic miRNAs and, sometimes, intronic miRNAs are transcribed by RNA polymerase II as independent units. The primary transcript (pri-miRNA) is capped and polyadenylated (Cai et al., 2004; Lee et al., 2004).

MicroRNA maturation begins in the nucleus, where the pri-miRNA is processed by a protein complex known as

[†]The miRBase Registry has adopted several rules for miRNA nomenclature and annotation. When a new miRNA is experimentally verified, a new numerical identifier is assigned, except in the case of orthologous miRNAs, which usually conserve the same number across species. The name includes a three- or four-letter prefix designating the species – for example, hsa-mir-001 (in *Homo sapiens*). The mature sequences are labeled 'miR', miRNA precursors or genes are labeled 'mir'. Related miRNA genes giving rise to mature miRNAs that differ at only one or two positions are indicated by the addition of a letter suffix to indicate subtypes (e.g. hsa-miR-10a and hsa-miR-10b); separate loci that give rise to identical mature miRNAs are marked by the addition of a numerical suffix (e.g. hsa-mir-16-1 and hsa-mir-16-2). Last, when mature miRNAs are generated from both complementary strands of the same hairpin precursor, the labels –5p (5′ arm) or –3p (3′ arm) are used to distinguish the two products.

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microprocessor, which contains the nuclear RNaseIII Drosha and its cofactor DGCR8/Pasha (Han et al., 2004; Han et al., 2006; Lee et al., 2003). Microprocessor action generates a precursor miRNA (pre-miRNA), a 60-70-nucleotide RNA that has a stem-loop structure and is rapidly exported to the cytoplasm by exportin-5 in a Ran-GTP-dependent manner. The mature miRNA(s) may reside in the 5′ arm or in the 3′ arm of the pre-miRNA stem; sometimes both arms generate mature miRNAs. Once in the cytoplasm, a second RNaseIII, Dicer, acts on the pre-miRNA to release a ~22-nucleotide miRNA duplex in which the mature miRNA is partially paired to a miRNA* present on the opposite stem strand. Usually, only the miRNA (mature miRNA) strand of the miRNA-miRNA* duplex is active and enters a specific protein complex, the RNA-induced silencing complex (RISC), to repress gene expression (Tang, 2005).

The mature miRNA guides RISC to regions of partial complementarity in the 3′ UTR of target mRNAs and triggers either their degradation or inhibition of translation, depending on the degree of complementarity between the miRNA and its target. In animal cells, post-transcriptional regulation by miRNA requires an mRNA sequence that is perfectly complementary to the 'seed sequence' (positions 2-7 of the mature miRNA). Various algorithms (http://www.microrna.org/; http://www.targetscan.org/; http://pictar.bio.nyu.edu/) have been developed for predicting miRNA target interactions. These indicate that each miRNA potentially regulates hundreds of target mRNAs (Lewis et al., 2005), and it seems plausible that most, if not all, mRNAs are post-transcriptionally regulated by miRNAs.

miRNAs are involved in development and differentiation

Given the importance of miRNAs in the development of lower organisms and their role in modulating the expression of numerous genes at the post-transcriptional level, it was not a surprise when they were found to be involved in development and differentiation in higher organisms too.

Notably, miRNAs have only been identified in multicellular organisms and are absent from unicellular organisms. They may thus be essential for organisms to differentiate different tissue types and/or maintain a particular differentiation state. Undifferentiated or poorly differentiated cells do not require miRNAs to survive. For example, mouse ES cells lacking *dicer*, and thus unable to develop mature miRNAs, are viable but fail to differentiate (Bernstein et al., 2003; Kanellopoulou et al., 2005). Several lines of evidence indicate that miRNAs are important for early and late development of vertebrates (Wienholds and Plasterk, 2005). For example, mouse and human ES cells express a specific set of miRNAs that are downregulated upon differentiation into embryoid bodies (Houbaviy et al., 2003; Suh et al., 2004). MiR-196 and possibly miR-10 are involved in regulation of *Hox* genes, which encode developmental transcription factors. Both genes are embedded in paralogous *Hox* clusters, they are expressed in patterns that are markedly reminiscent of those of *Hox* genes and miR-196a negatively regulates Hoxb8 (Mansfield et al., 2004; Yekta et al., 2004).

Some miRNAs appear to play a key role in the maintenance of a differentiation state. miR-181 is preferentially expressed in B-lymphocytes, and ectopic overexpression of miR-181 increases the fraction of B-lymphocytes and decreases the fraction of T-lymphocytes in mice (Chen et al., 2004). MiR-181 is also able to regulate homeobox proteins involved in myoblast differentiation (Naguibneva et al., 2006). Transfection of the muscle-specific miR-1 or the brain-specific miRNA miR-124 into human HeLa cells shifts the mRNA expression profile to that of muscle or brain cells, respectively (Lim et al., 2005). Mir-1 and miR-133 are transcribed together in a muscle-specific manner, and play distinct roles modulating myoblast proliferation and differentiation (Chen et al., 2006). Overexpression of miR-1 in the heart in transgenic mice results in defective proliferation and failure of ventricular cardiomyocyte expansion, which is indicative of premature differentiation of cardiomyocytes (Zhao et al., 2005). Recently, Lodish and colleagues have shown that MyoD activates the transcription of the miR-1 and miR-133 miRNAs, suggesting that these miRNAs are effectors of this muscle-specific transcription factor (Rao et al., 2006).

miR-122a is highly expressed in adult livers and its expression is upregulated during mammalian liver development (Chang et al., 2004). miR-143 is strongly expressed in adipose (fat) tissue and is upregulated during the differentiation of human pre-adipocytes into adipocytes (Esau et al., 2004). MicroRNAs are also involved in skin morphogenesis (Yi et al., 2006), and miR-134 functions in dendritic spine development (Schratt et al., 2006).

As small as they are, miRNAs thus seem to play key roles as control switches in development and cell differentiation (Table 1). Because these functions are essential in any specialized tissue, miRNA deregulation may affect differentiation, a biological process that is often upset in cancer cells.

miRNAs as tumor suppressor genes and oncogenes

The first evidence for miRNA involvement in human cancer

Table 1. miRNAs involved in mammalian development and cell differentiation

miRNA	Function	References
miR-196	Developmental patterning	Yekta et al., 2004
miR-181	B-lymphocyte differentiation	Chen et al., 2004
miR-181	Myoblast differentiation	Naguibneva et al., 2006
mir-133	Myoblast differentiation and proliferation	Chen et al., 2006
mir-122a	Liver-specific expression and regulation of cholesterol metabolism	Chang et al., 2004; Esau et al., 2006; Krutzfeldt et al., 2005
miR-1	Cardiomyocyte differentiation and proliferation	Chen et al., 2006; Lim et al., 2005
miR-375	Insulin secretion	Poy et al., 2004
mir-124	Induction of brain-specific expression profiles	Lim et al., 2005
miR-34	Dendritic spine development	Schratt et al., 2006
miR-143	Adipocyte differentiation	Esau et al., 2004

came from a study by Calin et al. (Calin et al., 2002). Examining a recurring deletion at chromosome 13q14 in the search for a tumor suppressor gene involved in chronic lymphocytic leukemia (CLL), this study found that the smallest minimal common region of deletion encodes two miRNAs, mir-15a and mir-16-1. Analysis of their expression in CLL samples and normal CD5$^+$ lymphocytes revealed that downregulation of miR-15a and miR-16-1, which share a primary transcript, is consistently associated with the deletion at chromosome 13q14. This suggested a role of miR-15a and miR-16-1 as tumor suppressor genes. Subsequent investigations have confirmed the involvement of miRNAs in the pathogenesis of human cancer. The putative tumor suppressive role of miR-15a and miR-16-1 was supported by the discovery in two CLL patients of a germ-line point mutation that results in reduced levels of mature miR-15a and miR-16-1 (Calin et al., 2005), and the idea was further strengthened by the demonstration that miR-15a and miR-16-1 negatively regulate the anti-apoptotic oncogene *BCL2* at a post-transcriptional level and induce apoptosis in the leukemic cell line MEG-01 (Cimmino et al., 2005).

miRNA deregulation can also operate in the opposite direction in cancer. The miR-17-92 family is the most studied example. This family includes fourteen homologous miRNAs, which are encoded by three gene clusters on chromosomes 7, 13 and X (Tanzer and Stadler, 2004). The cluster on chromosome 13 is amplified in human B-cell lymphomas, which leads to increased expression of various miRNAs. Interestingly, enforced expression of the miR-17-92 cluster acts together with MYC to accelerate tumor development in a mouse B-cell lymphoma model (He, L. et al., 2005); it thus acts as an oncogene. It has been reported that transcription of this cluster is also induced by MYC itself; oncogenic members of the miR-17-92 cluster may therefore act as MYC effectors.

Another important example of oncogenic miRNA is miR-155. Direct evidence of its oncogenic activity comes from studies of a transgenic mouse model overexpressing this miRNA in B lymphocytes (Costinean et al., 2006). As observed in human B-cell lymphomas, these mice exhibit preleukemic pre-B cell polyclonal expansion followed by B-cell malignancy, demonstrating the direct contribution of a miRNA to malignant transformation. Bi-directional relationships between oncogene activation and miRNA deregulation thus exist. By inhibiting oncogenes or functioning as their effectors, miRNAs could themselves act as tumor suppressor genes or oncogenes.

Mechanisms of miRNA deregulation in human cancer

Deregulation of miRNA expression levels emerges as the main mechanism that triggers their loss or gain of function in cancer cells. As described above, the activation of oncogenic transcription factors, such as MYC, represents an important mechanism for altering miRNA expression (O'Donnell et al., 2005). Genomic aberrations might also alter miRNA expression, since miRNA upregulation has been associated with genomic amplification (He, L. et al., 2005; O'Donnell et al., 2005), and miRNA downregulation has been associated with chromosomal deletions, point mutations and aberrant promoter methylation (Calin et al., 2002; Calin et al., 2005; Calin et al., 2004b; Saito et al., 2006).

Several examples of miRNAs whose expression is deregulated in human cancer have been reported. Downregulation of miR-143, miR-145 and members of the let-7 family has been described. The miR-143 and miR-145 genes, which reside in a genomic cluster similar to that encoding miR-15a/miR-16-1, are significantly downregulated in colon cancer tissue compared with colonic mucosa (Michael et al., 2003), and let-7 family members are downregulated in more than 50% of lung cancers (Takamizawa et al., 2004) as well as other neoplasms (Johnson et al., 2005).

An example of upregulated miRNA is miR-155, which lies in the only phylogenetically conserved sequence of *BIC*, a non-protein-coding gene that was discovered as site of proviral insertions in avian leucosis-virus-induced lymphomas (Tam et al., 1997). This miRNA and its primary transcript BIC are overexpressed in Hodgkin lymphoma, in pediatric Burkitt lymphoma and in diffuse large B-cell lymphoma (Eis et al., 2005; Kluiver et al., 2005; Metzler et al., 2004; van den Berg et al., 2003). Another example of upregulated miRNA is miR-21, a gene located at chromosome 17q23 in a chromosomal region frequently amplified in human cancer (Ehrbrecht et al., 2006; Sinclair et al., 2003), which is upregulated in human breast cancer and in glioblastoma (Chan et al., 2005; Ciafre et al., 2005; Iorio et al., 2005).

A peculiar mechanism linking miRNA to cancer was revealed by the analysis of a masked chromosomal translocation t(8;17) in a B-cell leukemia (Gauwerky et al., 1989), in which the mir-142 regulatory element is juxtaposed to the *MYC* proto-oncogene. Here, a miRNA regulatory element serves as a proto-oncogene activator. Although at present this mechanism appears to be unique, given the high levels and tissue-specific expression of several miRNAs it is a mechanism that should be further investigated in translocations identified in human cancer that are yet to be associated with culprit genes.

Expression of numerous miRNAs linked to cancer is modulated by environmental stimuli

Recent work indicates that a set of miRNAs is induced in response to hypoxia (Kulshreshtha et al., 2007). Hypoxia, a feature of the neoplastic microenvironment that leads to acidosis and toxic effects, requires genetic or adaptive cellular changes. It is significant that several of the miRNAs that respond to hypoxia are upregulated in various human cancers. Twenty of the 23 commonly upregulated miRNAs in cancer are induced by hypoxia (Kulshreshtha et al., 2007), which suggests that the response to this stress is one of the main mechanisms affecting miRNA expression in cancer cells. Interestingly, miR-21, one of the miRNAs that is induced by hypoxia and upregulated in cancer, possesses anti-apoptotic properties; its expression may therefore represent an adaptation to a hypoxic environment that favors cancer cell survival.

To dissect the mechanisms leading to miRNA deregulation in human cancer further, it will clearly be interesting to investigate miRNAs that are regulated by specific intracellular or extracellular stimuli that promote cell growth, death or differentiation. Current evidence indicates that miRNA expression is transcriptionally controlled by tissue-specific transcription factors. Changes in transcriptional programs will affect miRNA expression and, in turn, all downstream gene targets. As mentioned above, experimental evidence clearly

links miRNAs to cell differentiation. It will be interesting to determine whether expression of specific miRNAs is linked to cell cycle progression and/or p53-mediated responses to stress stimuli and how abnormalities in these pathways could affect miRNA expression in cancer cells. By dissecting the roles of miRNAs in these cancer-associated pathways, additional functions in various aspects of cell physiology should be revealed.

Pathways affected by miRNA deregulation in cancer

How exactly does downregulation of specific miRNAs lead to cancer? Mice carrying targeted deletions of miRNA-encoding genes have yet to be generated. However, various studies have begun to establish the molecular links between miRNA downregulation and malignant transformation. As mentioned above, miR-15a and miR-16-1, for example, act as regulators of the anti-apoptotic BCL2 oncoprotein. These miRNAs are encoded by genes located in a chromosomal region deleted in more than 50% of CLLs. In the leukemic cell line MEG-01, expression of miR-15a and/or miR-16-1 leads to BCL2 downregulation and increased apoptosis (Cimmino et al., 2005). Hence, the loss of expression of these miRNAs, by removing a control over BCL2 expression, may be relevant in the pathogenesis of human CLL. Indeed, BCL2 is highly expressed in CLL; however, unlike in follicular lymphoma, its activation is not associated with translocation to the IgH-encoding locus. BCL2 activation in CLL thus appears at least in part linked to the reduced expression of miR-15a and miR-16-1.

A molecular link between miRNA deregulation and cancer has also been established for members of the let-7 family, which regulate the RAS oncogenes. The 3′ UTR of the RAS mRNAs contains multiple binding sites for let-7 members, and forced expression of let-7 in human cancer cells reduces RAS protein levels (Johnson et al., 2005). Since let-7 is downregulated in several human cancers, this mechanism could lead to the activation of the RAS pathway. The importance of let-7 downregulation in cancer is supported by studies by Takamizawa et al. and Akao et al., who showed that let-7 can suppress the growth of A549 lung cancer cells and DLD-1 colon cancer cells in vitro (Akao et al., 2006; Takamizawa et al., 2004).

Mir-125a and miR-125b are downregulated in breast cancer (Iorio et al., 2005). These two miRNAs regulate the expression of the receptor tyrosine kinases ERBB2 and ERBB3 (Scott et al., 2007). Ectopic overexpression of miR-125a or miR-125b in SK-BR-3 cells induces impaired anchorage-dependent growth and reduced migration and invasion capacities, which is consistent with suppression of ErbB signalling.

The hypothesis that upregulated miRNAs act as oncogenes is also supported by work on in vivo models. We have previously mentioned the mir-155 transgenic mouse model that displays pre-leukemic pre-B-cell polyclonal expansion followed by B cell malignancy (Costinean et al., 2006) and the mir-17-92 cluster that acts together with MYC to accelerate tumor development in a mouse B-cell lymphoma model (He, L. et al., 2005). These lymphomas, differently from those arising in the MYC-only system, are also characterized by the absence of apoptosis, which suggests that various miR-17-92 family members regulate a pro-apoptotic gene. Interestingly, two miRNAs encoded by the cluster, miR-17-5p and miR-20a, negatively regulate the expression of E2F1, a transcription factor that promotes cell cycle progression but is also a strong inducer of apoptosis. The absence of apoptosis might thus be linked to the tight control of E2F1 by the miR-17 family. A more recent report, reveals that E2F1, E2F2, and E2F3 directly bind the promoter of the mir-17-92 cluster, activating its transcription, and miR-20a, a member of the mir-17-92 cluster, modulates the translation of the E2F2 and E2F3 mRNAs (Sylvestre et al., 2006). These results suggest the existence of a feed-back loop involving miR-20a and E2F that protects cells from apoptosis induced by excessive E2F expression. In human solid tumors, expression of the miR-17 cluster at chromosome 13 is upregulated in small-cell lung cancer, and ectopic overexpression of this cluster enhances lung cancer cell growth (Dews et al., 2006; Hayashita et al., 2005). Importantly, miR-17-92 upregulation leads to increased tumor angiogenesis, which is mediated by downregulation of the anti-angiogenic factors thrombospondin 1 (Tsp1) and connective tissue growth factor (CTGF), both predicted targets of the miR-17-92 microRNAs (Dews et al., 2006).

Transfection of cultured glioblastoma and breast cancer cells with anti-miRNA oligonucleotides (AMOs) aimed at miR-21 suppresses cell growth in vitro, and this is associated with increased apoptosis (Chan et al., 2005; Si et al., 2007). MiR-21 is overexpressed in colangiocarcinoma and its inhibition by these AMOs also increases sensitivity to the chemotherapeutic agent gemcitabine (see below) (Meng et al., 2006).

Lastly, miR-372 and miR-373 have been shown to cooperate with oncogenic RAS to transform primary human cells (Voorhoeve et al., 2006). Primary human cells undergo growth arrest and senescence in response to mitogenic signals from oncogenes such as RAS, through activation of the p53 pathway. This response is reversed by the presence of non-functional p53. Ectopic expression of miR372 or miR373 is thus sufficient to allow transformation in the presence of wild-type p53. Interestingly, miR372/miR373 bypasses the oncogene-activated p53 pathway, but not the p53-dependent DNA damage response, a characteristic of testicular germ cell tumors (TGCTs). The miR-371/373 cluster is indeed expressed in most TGCTs, in contrast to other types of tumors, which suggests a role in the development of these tumors.

Work on the models discussed above not only strongly supports a role for miRNAs in the pathogenesis of human malignancies, but also provides tools for testing miRNA-based therapeutic approaches; we discuss this further below.

How many microRNAs are involved in human cancer?

Evidence now indicates that the involvement of miRNAs in cancer is much more extensive than initially expected. Initial clues came from the observation that about 50% of known miRNA genes are located at sites of recurrent deletions or amplifications in human cancers (Calin et al., 2004b). More direct evidence for this has been provided by genome-wide expression studies.

Studies that investigated the expression of the entire microRNAome in various human solid tumors and hematologic malignancies have revealed differences in miRNA expression between neoplastical and normal tissues (Bottoni et al., 2007; Calin et al., 2005; Calin et al., 2004a; Chan et al.,

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Table 2. List of miRNAs deregulated in more than one human neoplasm

miRNA	Cancer vs normal cells	Types of neoplasm involved
let-7-a-2	↓	Breast cancer, lung cancer, liver cancer
let-7-a-3	↓	Breast cancer, liver cancer
let-7d	↓	Breast cancer, liver cancer
let-7f	↓	Breast cancer, liver cancer, thyroid cancer
miR-101	↓	Lung cancer, breast cancer, pituitary adenoma
miR-102	↑	Breast cancer, thyroid cancer
miR-124a	↓	Lung cancer, liver cancer, pituitary adenoma
miR-125a	↓	Lung cancer, breast cancer
miR-125b-1	↓	Breast cancer
miR-125b-1	↑	Gioblast, thyroid cancer
miR-125b-2	↓	Breast cancer
miR-125b-2	↑	Gioblast, thyroid cancer
miR-140	↓	Lung cancer, breast cancer, thyroid cancer, pituitary adenoma
miR-141	↓	Liver cancer, pituitary adenoma
miR-142	↓	Liver cancer, thyroid cancer
miR-143	↓	Lung cancer, breast cancer, liver cancer
miR-145	↓	Lung cancer, breast cancer, liver cancer
miR-146	↑	Lung cancer, thyroid cancer
miR-150	↑	Lung cancer, pituitary adenoma
miR-155	↑	Lung cancer, breast cancer, thyroid cancer, lymphoma
miR-15b	↓	Thyroid cancer, pitutary adenoma
miR-181a	↓	Liver cancer
miR-181a	↑	Thyroid cancer
miR-181b	↓	Glioblast, pituitary adenoma
miR-181c	↓	Liver cancer, glioblast
miR-181c	↑	Thyroid cancer
miR-191	↑	Lung cancer, breast cancer, pituitary adenoma
miR-192	↑	Lung cancer, pituitary adenoma
miR-198	↓	Lung cancer, glioblast
miR-199b	↓	Lung cancer, liver cancer
miR-202	↑	Breast cancer, thyroid cancer
miR-203	↑	Lung cancer, breast cancer
miR-21	↑	Breast cancer, lung cancer, thyroid cancer, glioblast
miR-210	↑	Lung cancer, breast cancer
miR-212	↑	Lung cancer, pituitary adenoma
miR-213	↑	Thyroid cancer, breast cancer
miR-219-1	↓	Lung cancer, thyroid cancer
miR-220	↓	Lung cancer
miR-220	↑	Thyroid cancer
miR-221	↑	Glioblast, liver cancer, thyroid cancer
miR-222	↑	thyroid cancer
miR-24-2	↑	lung cancer, thyroid cancer

Data were from the following references: Bottoni et al., 2007; Calin et al., 2005; Calin et al., 2004a; Chan et al., 2005; Ciafre et al., 2005; Eis et al., 2005; He, H. et al., 2005; Iorio et al., 2005; Kluiver et al., 2005; Kutay et al., 2006; Liu et al., 2004; Lu, J. et al., 2005; Mattie et al., 2006; Murakami et al., 2006; Pallante et al., 2006; Roldo et al., 2006; Volinia et al., 2006; Weber et al., 2006; Yanaihara et al., 2006.

2005; Ciafre et al., 2005; He, H. et al., 2005; Iorio et al., 2005; Kutay et al., 2006; Liu et al., 2004; Lu, J. et al., 2005; Mattie et al., 2006; Murakami et al., 2006; Pallante et al., 2006; Roldo et al., 2006; Volinia et al., 2006; Weber et al., 2006; Yanaihara et al., 2006). These studies show that each neoplasia has a distinct miRNA signature that differs from that of other neoplasms and that of the normal tissue counterpart. Moreover, it has become clear that some miRNAs are recurrently deregulated in human cancer (Table 2). In most cases, deregulation consistently acts in one direction, either upregulating or downregulating the miRNAs, which suggests that these miRNAs are likely to play a crucial role in tumorigenesis. There are, however, some unusual situations: for example, members of the miR-181 family are upregulated in some cancers, such as thyroid, pancreatic and prostate carcinomas (He, H. et al., 2005; Pallante et al., 2006; Volinia et al., 2006) but downregulated in others, such as glioblastomas and pituitary adenomas (Bottoni et al., 2007; Ciafre et al., 2005). Given the involvement of miR-181 in differentiation (Chen et al., 2004; Guimaraes-Sternberg et al., 2006; Naguibneva et al., 2006; Ramkissoon et al., 2006; Ryan et al., 2006), these apparent discrepancies might reflect the original differentiation status of the neoplastic tissues.

There are also examples of miRNAs deregulated in specific neoplasms: miR-122a, for example, a liver-specific miRNA, is downregulated in hepatocellular carcinoma (Kutay et al., 2006); miR-204 and miR-211 are specifically upregulated in insulinomas (Roldo et al., 2006). Again, tissue-specific aberrant expression of miRNAs might reflect the differentiation status of the cell.

These genome-wide studies indicate that members of the let-7 family, miR-145, miR-221, miR-21 and miR-155, are deregulated in several cancers. Since a variety of other evidence has connected these to human cancer, the expression work indeed appears to identify miRNAs relevant in cancer pathogenesis. Among the miRNAs identified, several have not yet been thoroughly investigated; our knowledge of this aspect of human cancer is thus still at an early stage.

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miRNAs as prognostic tools

Given the findings discussed above, aberrant miRNAs expression can clearly influence cancer phenotype. If so, specific miRNA expression signatures could reveal distinct subgroups of each cancer type. Indeed, this occurs in various cases. For example, higher levels of miR-155 are present in DLBCLs with an activated B-cell phenotype than with the germinal center phenotype. Because patients with activated B cell-type DLBCL have a poorer clinical prognosis, quantification of this miRNA may be clinically useful (Eis et al., 2005). Similarly, let-7 downregulation in non-small-cell lung cancer is associated with poor prognosis and reduced post-operative survival (Takamizawa et al., 2004). A specific expression signature consisting of 13 miRNAs is linked to disease progression in human CLL (Calin et al., 2005).

miRNAs as therapeutic molecules and targets

Several studies have established the potential usefulness of miRNA-based therapy in cancer. The induction of apoptosis by miR-15a and miR-16-1 in CLL (Cimmino et al., 2005), inhibition of growth of cancer cells by let-7 (Akao et al., 2006; Takamizawa et al., 2004), the reduced migration and invasion capacities induced by miR-125 in breast cancer cells (Scott et al., 2007) and the use of anti-miR-21 AMOs to elicit a pro-apoptotic response in glioblastoma and breast cancer cells are examples (Chan et al., 2005; Si et al., 2007). Significantly, the use of the anti-miR-21 AMOs increases susceptibility of colangiocarcinoma cells to gemcitabine (Meng et al., 2006), which suggests that miRNA-based therapy can be effectively combined with chemotherapy. The primary obstacle to translating this into effective therapies is the efficiency of delivery of miRNAs/AMOs to the target cells in vivo.

The development of oligonucleotides based on 2′-O-methyl or 2′-O-methylethyl residues or on nucleotides in which the ribose ring is 'locked' by a methylene bridge connecting the 2′-O atom with the 4′-C atom (locked nucleic acids, LNA-modifed nucleotides) has led to increased potency and stability and decreased toxicity of AMOs (Chan et al., 2005; Lecellier et al., 2005). Mixed DNA/LNA AMOs have been used to inhibit miR-21, which results in increased apoptotic death in glioblastomas cells (Chan et al., 2005). LNA-based oligonucleotides have been shown to be non-toxic at dosages of less than 5 mg/kg/day in mice and these produce anti-tumor effects in vivo (Fluiter et al., 2005; Fluiter et al., 2003). AMOs may therefore be feasible for in vivo cancer therapy. More recently, it was demonstrated that intravenous injection of cholesterol-conjugated AMOs in mice significantly represses miR-16, miR-122, miR-192 and miR-194 activity in several organs. Furthermore, since the anti-miR-122 AMO affects cholesterol biosynthesis, reduced levels of plasma cholesterol are also observed in treated mice (Krutzfeldt et al., 2005).

At present, there are no reports of the use of miRNAs for in vivo anti-cancer therapy. However, the development of approaches for in vivo delivery of short interfering RNA (siRNA) and short heteroduplex RNA (shRNA) (Devi, 2006) to silence single target genes has established technical approaches also useful for miRNA delivery. Anti-cancer approaches based on systemic delivery of siRNA/shRNA in preclinical models have made use of viral vectors, liposomes and nanoparticles (Abbas-Terki et al., 2002; Lu, P. et al., 2005; Schiffelers et al., 2004; Tong, 2006). It is likely that similar approaches could be tested for the use of miRNAs as therapeutic molecules. The advantage of miRNAs over siRNA/shRNAs is the possibility that they can affect multiple targets with a single hit. Similarly to miRNAs, siRNA/shRNAs may also interact with multiple targets; however, this effect is seen as a disadvantage, because off-target effects are largely unpredictable. For miRNAs, restoration of expression in cancer cells is likely to re-establish the whole network of physiological interactions.

At present, the availability of only a few 'engineered' animal models for cancer-associated up- or downregulated miRNAs significantly limits pre-clinical in vivo studies. It would be interesting to examine mouse neoplasms and their patterns of miRNA deregulation to verify the similarities with their human counterparts. Indeed, because mouse and human hepatocarcinomas appear to share similar sets of deregulated miRNAs (Kutay et al., 2006), it is possible that spontaneous or induced neoplasms in mice may already represent suitable models for testing the safety and efficacy of miRNAs or AMOs as therapeutic agents.

Conclusions

miRNAs have erupted into the field of cancer research. Strong evidence of their consistent deregulation in human neoplasms through mechanisms linked to genomic alterations and oncogene activation indicate that they may act as oncogenes or tumor suppressor genes. Several lines of evidence support their role in biological processes that are aberrant in cancer, such as differentiation and apoptosis. In addition, the demonstration that there are links to gene products, such as BCL2, MYC and RAS, that regulate pathways associated with malignant transformation establishes a basis for understanding mechanisms linking miRNA deregulation to specific molecular pathways.

Besides gene expression studies, which have been methodically applied to a variety of human malignancies, all the other studies have only yielded proofs of principle. These now need to be extended to define in full the role of miRNAs in cancer and the mechanisms connected to their deregulation. Additional studies are needed to identify and validate new targets of miRNAs and establish the relationships with the molecular pathways involved in cancer. Finally, assessment of the potential for miRNAs as diagnostic markers or therapeutic molecules/targets is still only beginning. The development of animal models will certainly help us further establish the role of miRNAs in tumorigenesis and develop tools useful for in vivo testing of anti-cancer AMOs and miRNAs.

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