Human Molecular Genetics, 2007, Vol. 16, Review Issue 1 ***R106–R113***
doi:10.1093/hmg/ddm056

miRNAs in cancer: approaches, aetiology, diagnostics and therapy

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Received February 22, 2007; Revised and Accepted March 2, 2007

MicroRNAs (miRNAs) are causing tremendous excitement in cancer research. MiRNAs are a large class of short non-coding RNAs that are found in many plants, animals and DNA viruses and often act to inhibit gene expression post-transcriptionally. Approximately 500 miRNA genes have been identified in the human genome. Their function is largely unknown, but data from worms, flies, fish and mice suggest that they have important roles in animal growth, development, homeostasis and disease. MiRNA expression profiles demonstrate that many miRNAs are deregulated in human cancers. MiRNAs have been shown to regulate oncogenes, tumour suppressors and a number of cancer-related genes controlling cell cycle, apoptosis, cell migration and angiogenesis. MiRNAs encoded by the *mir-17-92 cluster* have oncogenic potential and others may act as tumour suppressors. Some miRNAs and their target sites were found to be mutated in cancer. MiRNAs may have great diagnostic potential for human cancer and even miRNA-based cancer therapies may be on the horizon.

INTRODUCTION

Over the last 10 years a small RNA revolution has swept biology. In 1998 interference RNA (RNAi) was discovered as an experimental tool by Andy Fire and Craig Mello, a finding that was awarded with the 2006 Nobel Prize for Physiology or Medicine (1). Although the biology of RNAi is still not understood, it has become a powerful experimental tool and is currently being developed for human gene therapy (2). During a similar timeframe and linked in some aspects to RNAi, microRNAs (miRNAs) were discovered as a new class of regulatory RNAs in animals, plants and viruses (3). miRNAs are transcribed from endogenous genes as long, primary RNA transcripts and are processed to their mature form: a single-stranded RNA with a length of approximately 22 nucleotides, indistinguishable from a small-interfering RNA (siRNA), the mediator of RNAi. In animals these long RNA precursors (pri-miRNAs) (4) are processed in the nucleus by the RNase III enzyme Drosha and Pasha/DGCR8 to form the approximately 70-base pre-miRNAs (5–9). Pre-miRNAs are exported from the nucleus by Exportin-5 (10), processed by the RNase III enzyme Dicer and incorporated into an Argonaute-containing silencing complex (RISC) (11). miRNAs are thought to regulate gene expression post-transcriptionally by forming Watson-Crick base pairs with target mRNAs. Their mechanism of action is still under debate, but likely includes inhibition of translation and mRNA degradation (12). In animals, most miRNAs are thought to form imperfect base pairs with their target mRNA(s) and these interaction sites are enriched in 3′ un-translated regions (3′-UTRs) (3). As a consequence, miRNA target identification using computational approaches is non-trivial (13). The public database for miRNAs, miRBase release 9.1, currently lists 474 human microRNAs (14,15) and estimates for the total number of human microRNAs range from over one thousand (16) to tens of thousands (17). Although miRNAs have only been studied intensely for the last 5 years, important functions for miRNAs in animal development and, potentially, human disease, have already emerged (18).

This review focuses on the approaches and current experimental evidence for the involvement of miRNAs in the aetiology of human cancer and the potential for miRNAs in human cancer diagnostics and therapy.

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OF WORMS AND FLIES

Genetic analysis of the timing of development in the nematode *Caenorhabditis elegans* (19–21) led to the cloning of the first miRNA, *lin-4* miRNA (22), and the identification of the first miRNA target, the *lin-14* mRNA (23). The developmental timing or heterochronic pathway regulates stage-specific processes during *C. elegans* larval development (24). The *lin-4* miRNA and the second miRNA to be identified, *let-7* miRNA, control hypodermal cell-fate decisions during larval development (25). Three additional *C. elegans let-7*-like miRNAs, miR-48, miR-84 and miR-241 also act in the control of developmental timing (26,27). All five of these *C. elegans* miRNA genes are required for hypodermal stem cell lineages to undergo stage-specific terminal differentiation. As a consequence, loss-of-function mutations in any of these miRNAs lead to excess cellular proliferation. Based on these observations alone, *lin-4* or *let-7* family miRNAs may be thought of as candidate tumour suppressors. Subsequently, both miRNA families were found to be conserved in mammals (28,29) and the human *let-7* miRNA has been directly implicated in cancer (30). The first microRNA whose function was studied in *Drosophila* is encoded by the *bantam* locus, which had previously been identified in a screen for de-regulated tissue growth (31,32). The *bantam* microRNA stimulates cell proliferation and reduces programmed cell death. *Bantam* directly regulates the pro-apoptotic gene *hid*. A second *Drosophila* microRNA, miR-14, also limits programmed cell death (33). Although no human orthologues of *bantam* or miR-14 have been identified yet, their role in tissue growth and apoptosis emphasize the potential roles for miRNAs in biological processes of relevance to human cancer.

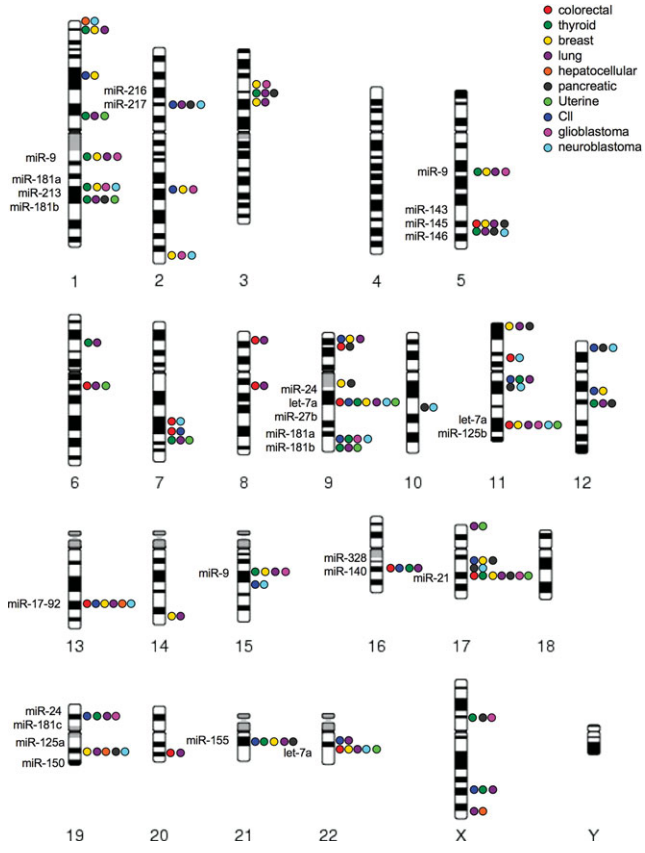

Figure 1. An miRNA cancer map. Chromosome positions of miRNAs implicated in human cancer are shown as coloured dots. Each dot represents a single miRNA or an miRNA cluster. Colours refer to tumour tissue type, as indicated. Only miRNAs, whose expression levels were found to be significantly altered in tumours versus normal tissues in at least two tissue types are shown. miRNAs identified in at least four different tumour types are also indicated by their name. Data were collated from the studies of primary human tumours of the colorectum (35,92,93), thyroid (60,94,95), breast (62,96), lung (97), liver (98), pancreas (99–101), uterus (52) and in chronic lymphocytic leukaemia (CLL) (37,64,102), glioblastoma (103) and neuroblastoma (104).

MICRORNAS EXPRESSION PROFILING

The first evidence for a direct link between miRNAs and human cancer came from the observation that two microRNA genes, *mir-15* and *mir-16* are located in a 30 kb region on chromosome 13 that had been found deleted in chronic lymphocytic leukaemia (CLL) cases, and that miR-15 and miR-16 expression is often reduced in CLL (34). A second study found that miR-143 and miR-145 expression levels were reduced in adenomatous and cancer stages of colorectal neoplasia (35). Both studies were focused on a small number of miRNAs and based on miRNA cloning and northern blotting approaches. Subsequent development of a number of miRNA microarray technologies coincided with an increased number of miRNA expression studies in human cancer (36–41). Crucially, quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) for mature miRNAs have also become available for the analysis of small tissue samples and microarray miRNA validation (42,43). A current map of all miRNA loci implicated in at least two different human cancers through expression profiling lists 56 such loci (Fig. 1). However, the number of miRNAs implicated in human cancer by expression profiling will still increase likely substantially for two reasons: first, the recent availability of commercial miRNA profiling platforms will widen access to these tools and secondly, the number of known human miRNAs is still increasing, rendering even the most recently published expression studies incomplete. Some general themes from the expression profiles published to date are emerging. For example, a study of 334 primary human tumours and tissues interrogating the expression of 217 miRNAs revealed that miRNA expression profiles contain lineage-specific information, may classify even poorly differentiated tumours, and that many miRNAs are down-regulated in primary tumours when compared with normal tissues (40). The notion that a common set of miRNAs may be deregulated in many tumour types is also supported by the finding that a large number of miRNAs have been implicated in at least two distinct tumour types (Fig. 1). It is tempting to speculate that these miRNAs share anti-tumourigenic properties. It is unknown how miRNA expression in tumours may be de-regulated. The development of *in situ* approaches to directly visualize miRNAs in human tumour cells using oligonucleotide probes, which have been successful in plants, flies and fish (44–46), may help address the question if miRNA expression is altered stochastically or co-ordinately, is cell-specific or tumour-wide.

Table 1. miRNAs that may regulate cancer-related genes

Gene	Gene summary	Cell function	miRNAs	References
AIB1	Amplified in breast cancer 1		miR-17-5p	(62)
AT1R	Angiotensin receptor 1	Angiogenesis	miR-155	(105)
BCL2	B-cell CLL/lymphoma 2	Apoptosis	miR-21	(57,58)
			miR-15a	
			miR-16-1	
BCL6	B-cell CLL/lymphoma 6	Cell cycle	miR-127	(56)
E2F1	E2F transcription factor 1	Cell cycle	miR-17-5p	(65)
		Apoptosis	miR-20a	
E2F3	E2F transcription factor 3	Cell cycle	miR-34a	(54)
FOS	FBJ osteosarcoma virus oncogene homolog		miR-7b	(51)
HMGA2	High mobility group AT-hook 2		*let-7* family	(53)
				(52)
HOXB8	Homeobox B8		miR-196a	(106)
KIT	KIT oncogene		miR-221	(59,60)
			miR-222	(61)
			miR-146	
LATS2	Large tumour suppressor homolog	Cell cycle	miR-372	(48)
			miR-373	
NFI-A	Nuclear factor I/A		miR-107	(107,108)
			miR-233	
PLAG1	Pleomorphic adenoma gene 1		miR-26a	(50)
PTEN	Phosphatase and tensin homolog	Cell cycle	miR-21	(49)
		Cell migration		
RAS	Harvey rat sarcoma viral oncogene homolog		*let-7* family	(30)
RB	Retinoblastoma	Cell cycle	miR-106a	(50)
TCL1	T-cell leukaemia/lymphoma 1A	Apoptosis	miR-29	(55)
			miR-181	
TGFBR2	Transforming growth factor-beta receptor 1		miR-20a	(50)
TSP1	Thrombospondin 1	Angiogenesis	miR-17-92	(69)

Italics indicates human oncogenes. Bold indicates human tumour suppressors. Only predicted targets for which there is some experimental verification are listed.

FUNCTIONAL STUDIES

Two decades ago cell-based transformation assays identified the first human oncogenes and tumour suppressors (47). More recently, a cell-based screen identified miR-372 and miR-373 out of 197 miRNAs as putative oncogenes in a RAS co-operation assay (48). This study implicated miR-372 and miR-373 in the p53 pathway with the tumour suppressor *LATS2* as a possible direct target and suggested a potential role for these miRNAs in testicular germ cell tumours (Table 1). A number of other studies have taken specific miRNAs identified in expression profiling experiments forward to carry out functional analyses. For example, miR-21 was found to be over-expressed in malignant cholangiocytes and miR-21 expression was found to down-regulate the tumour suppressor PTEN in these cells (49). In a separate study the microRNAs miR-20 and miR-106a were found to be able to regulate the tumour suppressors TGFBR2 and RB, respectively (50). All of the above, and work on the miR-17 to -92 cluster of miRNAs that will be discussed in the next section, suggest that a number of miRNAs may have oncogenic potential. However, several miRNAs have been proposed to act as tumour suppressors. The first of these included members of the *let-7* family, which were reported to regulate the expression of the *RAS* oncogene in *C. elegans* and in human cells (30). The same study found *let-7* to be down-regulated in lung tumours and its expression anti-correlated with that of *RAS*. This observation was particularly intriguing given the functional studies of the *let-7* miRNA in *C. elegans*. The *let-7* family was subsequently found to be de-regulated in a large number of tumour types (Fig. 1) and the oncogenes HMGA2 and FOS have been added to its list of putative targets (51–53). Other oncogenes that may be targeted directly by miRNAs include AIB1, BCL2, BCL6, E2F3, KIT and TCL1 (54–62). Interestingly, miR-21 was shown to be able to regulate the oncogene PTEN in cholangiocytes (49) and the tumour suppressor BCL2 in the breast cancer cell line MCF-7 (57), suggesting that miR-21 may act as a tumour suppressor or oncogene depending on cellular context. All cancer-related genes that may be targeted by one or more miRNAs are listed in Table 1.

THE MIR-17-92 MIRNA CLUSTER

If the *let-7* miRNA is the best-studied candidate tumour suppressor miRNA, the *mir-17-92* miRNA polycistron is the best-studied miRNA with oncogenic potential and was named 'OncomiR-1'. The *mir-17-92* cluster (*c13orf25*) is part of a region on chromosome 13 amplified in malignant lymphoma including B-cell lymphoma (63). Using a well-established mouse model of B-cell lymphoma in which haematopoietic stem cells over-expressing the oncogenic transcription factor Myc are used to generate mosaic animals it was demonstrated

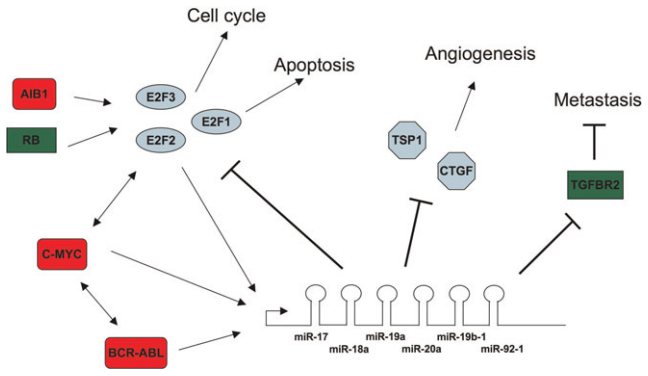

Figure 2. Interaction map of the oncogenic miR-17-92 cluster on chromosome 13 q13.3. Proteins in red and green are products of known human oncogenes and tumour suppressors, respectively (50,62,65–71).

that mice mosaic for cells over-expressing *Myc* and the *mir-17-92* cluster developed tumours earlier than mice with cells over-expressing Myc alone (64). Furthermore, *Myc* and *mir-17-92* cluster-induced tumours were more aggressive with higher mitotic indices than Myc-only induced tumours (64). An independent study demonstrated that Myc directly binds to and regulates the transcription of the *mir-17-92* cluster in cell culture and that the miRNAs of this cluster regulate the expression of E2f1 (65). These two studies suggested a complex network of regulation whereby MYC, which is a positive regulator of E2F1 transcription, dampens the E2F1 response through the miRNAs of the *mir-17-92* cluster (Fig. 2). However, the picture is likely more complex involving several E2F proteins (E2F1, E2F2, E2F3), feedback loops from E2F to MYC and from E2F to the *mir-17-92* cluster and affects angiogenesis and tumour metastases by targeting TSP1, CTGF and TGFBR2 (50,62,65–71).

MICRO-RNA BIOGENESIS

The de-regulation of many miRNAs in human cancer raises the question of how these changes are orchestrated. The previous section possibly offers a simple explanation for the *mir-17-92* cluster, which is directly regulated by the oncogene *Myc* (65). In some cases, changes in miRNA expression may therefore reflect changes in the upstream transcriptional network. In the case of *mir-127*, transcription appears to be regulated at the level of histone acetylation and DNA methylation (56). Recently, similar observations were made for the *mir-124a* and the *let-7a-3* loci (72,73). However, this may not be a general phenomenon (74). In contrast, miRNA expression may also be regulated post-transcriptionally. This possibility was first noted, when it was shown that the *let-7* pre-miRNA in sea urchins is present throughout development, whereas the mature form is stage-specific (28). Similar developmental regulation of miRNA processing was observed in the mouse (75). Moreover, the mouse pre-miRNA miR-138-2 was found to be expressed ubiquitously, but only processed to its mature form in specific tissues (76). As most miRNA expression profiles are focussed on the mature miRNA, deregulation in cancer may be a reflection of changes in post-transcriptional processing at the level of the pri-miRNA or pre-miRNA. Indeed, in mouse embryos *let-7* family pri-miRNAs accumulate prior to processing by the Drosha (77). The same study also claimed that post-transcriptional regulation of miRNAs in cancer is common as a cross-platform analysis of miRNA and mRNA expression profiles of primary human tumours lacked correlation (40,77,78). The mechanism(s) controlling differential miRNA processing is currently unknown, but may prove important for understanding the roles of miRNAs in cancer in future. However, given that many miRNAs are deregulated in cancer, it is possible that alterations in the core biogenesis machinery contribute to cancer. Indeed, elevated DICER levels have been reported in prostate adenocarcinoma (79) and Burkitt's lymphoma (80) and reduced DICER levels have been reported in lung cancer (81).

GERMLINE MUTATIONS

Given the data implicating miRNAs in cancer reviewed here, one might expect germline mutations in miRNAs to be involved in cancer pre-disposition. Indeed, single nucleotide polymorphisms (SNPs) in the primary transcripts of miR-15a and mIR-16-1 may be linked to CLL (82). But the net should be cast much wider to include miRNA targets. Our current understanding of miRNA interaction with target mRNAs suggests that a single 3′-UTR mutation might make or break the miRNA interaction (13). The importance of mutations in miRNA binding site for inheritable traits has already been demonstrated in the case of Texel sheep, where a quantitative trait locus maps to a mutation in the 3′-UTR of the muscle-specific gene *GDF8*, which renders it a target for the miRNAs miR-1 and miR-206 (83). miRNA target site mutations have also been shown to be of potential importance for human disease as a mutation in a putative miR-189 binding-site in human SLITRK1 may be linked to Tourette's syndrome (84). Interestingly, SNPs in the binding sites for the miRNAs miR-146, miR-221 and miR-222 in the oncogene KIT may be linked to papillary thyroid carcinoma (60). However, a recent study identified a number of SNPs in primary miRNA sequences, one in the pre-miRNA for miR-26-a1, and none in mature miRNAs in a panel of 91 human cancer cell lines (74). None of the miRNA-associated SNPs resulted in changes in miRNA expression or processing (74).

DIAGNOSTICS

The body of expression data for miRNAs in cancer available to date suggests that miRNAs may have diagnostic potential. However, while initial expression studies focused on comparing normal tissues to tumours, to gauge diagnostic potential it will be more important to correlate miRNA expression with tumour subtypes or clinical parameters. A number of studies show promise in this regard. For example, a qRT-PCR-based study identified subsets of miRNAs that distinguish ErbB2-positive from ErbB2-negative and ER-positive from ER-negative breast cancers from biopsies (85). If miRNA expression data can be used to build discriminators with clini-

cal value, miRNAs have clear advantages over mRNAs: they are long-lived *in vivo* (86) and very stable *in vitro*.

THERAPY

Given the emerging evidence of miRNAs with oncogenic or tumour suppressor activities, it is important to seek routes to interfere with miRNAs and to develop these as novel cancer therapies. In the case of the oncogenic *mir-17-92* cluster it might therefore be of interest to specifically knockdown the expression of all miRNAs derived from this cluster. In mammalian cell culture, 2-*O*-methyl oligoribonucleotides (2-*O*-Me-RNA) have been used to specifically down-regulate miRNAs (87). Cell-permeable forms of these 2-*O*-Me-RNAs, called 'antagomirs' were successful in down-regulating several mouse miRNAs in a number of mouse tissues following intravenous injection *in vivo* (88). It remains an open question whether these or similar strategies will allow us to deliver anti-miRNA cancer therapies. One complication that may make the task more difficult is the potential redundancy of miRNAs: would targeting *mir-17* be sufficient or would all miRNAs of the *mir-17-92* cluster have to be targeted? In contrast, for miRNAs that act as tumour suppressors it may be of interest to develop *in vivo* expression systems. As miRNAs are chemically identical to siRNAs ongoing efforts to deliver siRNAs as RNAi-based anti-cancer therapies, if successful, should provide suitable vehicles for the delivery of miRNAs (2). In fact, some methods of siRNA delivery make use of the miRNA biogenesis pathway to increase efficiency (2). Even if the delivery problem may be solved, one major obstacle to the efficacy of siRNA- or miRNA-based therapies may be the potential of off-target effects (89,90). Finally, inhibiting miRNA expression globally by interfering with their biogenesis may have therapeutic potential just as inhibiting global chromatin-modifying enzymes such as histone deacetylases (HDACs) is proving effective in some types of cancer (91).

CONCLUSIONS

The last 6 years have demonstrated the importance of miRNAs in biology. The evidence for roles of miRNAs in human disease, in particular cancer, is overwhelming. Diagnostic potentials for miRNAs have been identified and we expect that clinical diagnostic trials will test their efficacy soon. There are clear opportunities for miRNA-based anti-cancer therapeutics. Although initially in the shadow of RNAi, miRNAs have become the brightest star of the small RNA revolution.

ACKNOWLEDGEMENTS

We thank Ines Alvarez-Garcia for help with the manuscript. E.A.M. is a Cancer Research UK Programme Grant Holder (C13474/A4613).

Conflict of Interest statement. None declared.

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