Causes and consequences of microRNA dysregulation in cancer

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Abstract | Over the past several years it has become clear that alterations in the expression of microRNA (miRNA) genes contribute to the pathogenesis of most — if not all — human malignancies. These alterations can be caused by various mechanisms, including deletions, amplifications or mutations involving miRNA loci, epigenetic silencing or the dysregulation of transcription factors that target specific miRNAs. Because malignant cells show dependence on the dysregulated expression of miRNA genes, which in turn control or are controlled by the dysregulation of multiple protein-coding oncogenes or tumour suppressor genes, these small RNAs provide important opportunities for the development of future miRNA-based therapies.

CpG island
A sequence of at least 200 bp with more CpG sites than would be expected for its GC content. These sequences are often GC rich, are typically undermethylated and are found upstream of many mammalian genes.

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For the past two and a half decades it has been thought that cancer is caused by genetic and/or epigenetic alterations to protein-coding oncogenes and tumour suppressor genes. These alterations result mainly from somatic genetic events[1] that occur over long periods of time, particularly in solid malignancies, such as lung, breast, prostate and gastrointestinal cancers. These findings have informed the development of novel (targeted) therapies that are based on the specific genetic alterations that are involved in cancer pathogenesis. For example, in the case of chronic myeloid leukaemia (CML), the disease has been shown to be caused by a t(9;22) chromosome translocation that leads to a fused breakpoint cluster region (*BCR*)–*ABL1* gene with dysregulated *ABL* oncogene tyrosine kinase activity[2,3]. Novartis investigators have developed an inhibitor of the *ABL* tyrosine kinase called imatinib (Glivec; Novartis), which induces complete remission in ~95% of CML patients in the chronic phase[4,5]. Over the past few years, other drugs have been developed that affect (for example, by inhibiting angiogenesis) the activity of oncogenic proteins or the function of genes that facilitate tumour growth and/or survival. Such drugs are being used in patient treatment and clinical trials[1].

Although it is clear that tumours are initiated, for the most part, by somatic genetic alterations[1], it is also evident that many tumours show alterations in the expression of tumour suppressor genes because of epigenetic alterations — such as the methylation of CpG islands in their promoters — that lead to loss of function[6]. Therefore, therapies directed towards the reversal of the epigenetic changes that occur in various malignancies have been developed. For example, azacitidine (Vidaza; Celgene) and decitabine (Dacogen; Eisai) have been used to reactivate tumour suppressor genes that have been silenced by the methylation of promoter CpG islands[7]. Histone deacetylase (HDAC) inhibitors have also been used to reactivate the expression of silenced genes[8].

Although progress has been made in identifying the genetic and epigenetic causes of cancer, and therefore in identifying targets for therapy, several challenges remain. For example, although targeted therapies that are based on the identification of oncogenic mutations with causal roles in cancer have been developed, the treatment of malignancies that are initiated through the loss of function of tumour suppressor genes is more difficult, as the lost gene function must be replaced in all the cancer cells. The identification of additional alterations that cause or contribute to malignancy is a high priority.

In 1993, Victor Ambros and colleagues discovered a gene, *lin-4*, that affected development in *Caenorhabditis elegans* and found that its product was a small non-protein-coding RNA[9]. After these seminal findings, the cloning and characterization of small, 20–22 nucleotide-long members of the non-protein-coding RNA family have led to the identification of ~1,000 microRNAs (miRNAs). miRNAs are generated by a mechanism that typically involves the transcription of a long precursor (pri-miRNA), which is then processed to produce a second precursor (pre-miRNA) of 60–100 nucleotides in length. The pre-miRNA is transported to the cytoplasm and a second processing step is carried out by an endoribonuclease of the RNase III family, which results in the final miRNA products (for a review, see REF. 10).

Box 1 | **Defining the contribution of microRNAs to cancer pathogenesis**

A straightforward approach to defining the contribution of microRNAs (miRNAs) to cancer pathogenesis is through expression profiling, which can be done by various methods, including microarrays and reverse transcription followed by PCR (RT–PCR). In general it is good practice to carry out profiling by microarray and validation by RT–PCR[12]. Profiling provides important information concerning the miRNAs that are dysregulated in a specific malignancy versus its normal tissue counterpart. It is important to discover the mechanisms of dysregulation and the potential targets of the dysregulated miRNAs. If the mechanism of dysregulation involves genetic alterations at an miRNA locus — for example, a deletion or a mutation that affects the processing of the miRNA or the amplification of an miRNA locus — it provides strong evidence for a contribution of that miRNA or miRNA cluster to cancer pathogenesis. If such genetic alterations occur early during the multistep process of malignant transformation in a large fraction of tumours, it suggests a role for that miRNA in the initiation of the process, providing a valuable target for therapeutic intervention.

In the cytoplasm, miRNA duplexes are unwound and the mature strand is incorporated into RNA-induced silencing complex. Following binding to partially complementary sites, usually in the 3′ UTRs of mRNAs, miRNAs cause an inhibition of translation and some degree of degradation of the target mRNA (for reviews, see REFS 11,12). Therefore, miRNAs are negative regulators of gene expression. They have been found to regulate over 30% of mRNAs and have roles in fundamental processes — such as development, differentiation, cell proliferation, apoptosis and stress responses — in a range of organisms, including *C. elegans*, plants, *Drosophila melanogaster* and mammals (including humans)[10,13].

Over the past few years, many miRNAs have been implicated in various human cancers (BOX 1). Both losses and gains of miRNA function have been shown to contribute to cancer development through a range of mechanisms. Here, I discuss the causes and consequences of miRNA dysregulation in cancer and the opportunities that this knowledge has provided for the development of new cancer therapeutics.

The discovery of microRNAs as cancer genes

During our attempts to clone a tumour suppressor gene at 13q14, a chromosomal region that is frequently lost in chronic lymphocytic leukaemia (CLL; the most common human leukaemia[14]), we found that all of the protein-coding genes that were present in the region of interest were not specifically altered in CLL, which suggested that they were not involved in the disease. We took advantage of a rare chromosome translocation involving 13q14 in CLL and mapped the breakpoint precisely to the region of loss. We also found a CLL case with a very small deletion of ~30 kb at 13q14 (REF. 15). Interestingly, the translocation breakpoint was located in the deleted region in the second case. These results suggested that the CLL suppressor gene was located in this small genomic region, in which there were no protein-coding genes. However, we found two miRNA genes, *miR-15a* and *miR-16-1*, in the deletion. The translocation breakpoint cut the precursor of these two miRNA genes, which reside in the same polycistronic RNA. This indicated that the loss of *miR-15a* and *miR-16-1* was the target of 13q14 deletion in CLL pathogenesis[15]. Following this discovery, we observed the loss of *miR-15a* and *miR-16-1* in ~70% of CLLs[15]. As most indolent CLLs have lost *miR-15a* and *miR-16-1*, we concluded that this loss must be an early event — perhaps the initiating event — in the pathogenesis of the indolent form of CLL. Indolent CLLs frequently progress to an aggressive disease, and a minority of CLL cases are aggressive at presentation.

These early results suggested that *miR-15a* and *miR-16-1* can function as tumour suppressor genes in CLL, as their loss is associated with the development of the indolent form of CLL[15]. This finding prompted us to map the chromosomal location of all known miRNA genes, which resulted in another surprising finding; many miRNA genes are located in chromosomal regions that are frequently involved in chromosomal alterations, such as deletions or amplifications, in many types of human cancers. Extensive studies of these cancers have failed to find the activation of oncogenes or the loss of tumour suppressor genes, which suggests that the role of miRNAs in cancer could extend far beyond CLL[16]. Therefore, the mapping of miRNA genes to specific chromosomal locations provided important clues for the possible roles of these miRNA genes in cancer[16] (FIG. 1).

These clues were supported by the expression profiling data of many different types of tumours (BOX 1). For example, members of the *let-7* family of miRNAs that map to chromosome regions that are deleted in multiple tumours were found to be lost in multiple different malignancies, including those of lung, breast, urothelial, ovarian and cervical cancers. Interestingly, an additional locus at 3q26 carries homologues of *miR-15a* and *miR-16-1*: *miR-15b* and *miR-16-2*. These miRNAs are expressed at low levels in normal CD5-positive B cells[15]; however, they could be involved in other human malignancies. Similarly, *let-7* family members map to different human chromosomes[16], are differentially expressed in different tissues and are involved in different human tumours[17,18]. The *miR-17–92* cluster, which maps to a region that is amplified in lymphoma, was found to be overexpressed in many different tumours[19]. Consistent changes in miRNA expression were also observed in tumours without evident specific cytogenetic abnormalities, suggesting that pathways that are commonly dysregulated in human cancer could directly affect and dysregulate miRNA expression[19]. A corollary to this interpretation is that miRNAs could be the downstream targets and effectors of pathways that regulate important processes, such as development, proliferation, differentiation, apoptosis and stress responses[19]. If crucial pathways can be dysregulated by alterations in multiple genes, many of which may not be easily targetable by small molecules and many of which may be lost, a reasonable speculation is that their downstream miRNAs may become the suitable, and perhaps optimal, targets for therapeutic intervention. Therefore, if the dysregulation of a crucial pathway results in the loss of expression of a specific miRNA(s), this miRNA(s) could be used therapeutically. If it results in the overexpression of a specific miRNA(s), complementary (antisense) molecules — such as anti-miRNAs — could be used for treatment.

RNA-induced silencing complex
An Argonaute protein–small RNA complex that inhibits the translation of target RNAs through degradative or non-degradative mechanisms.

Indolent
In medical terms, slow to develop or heal.

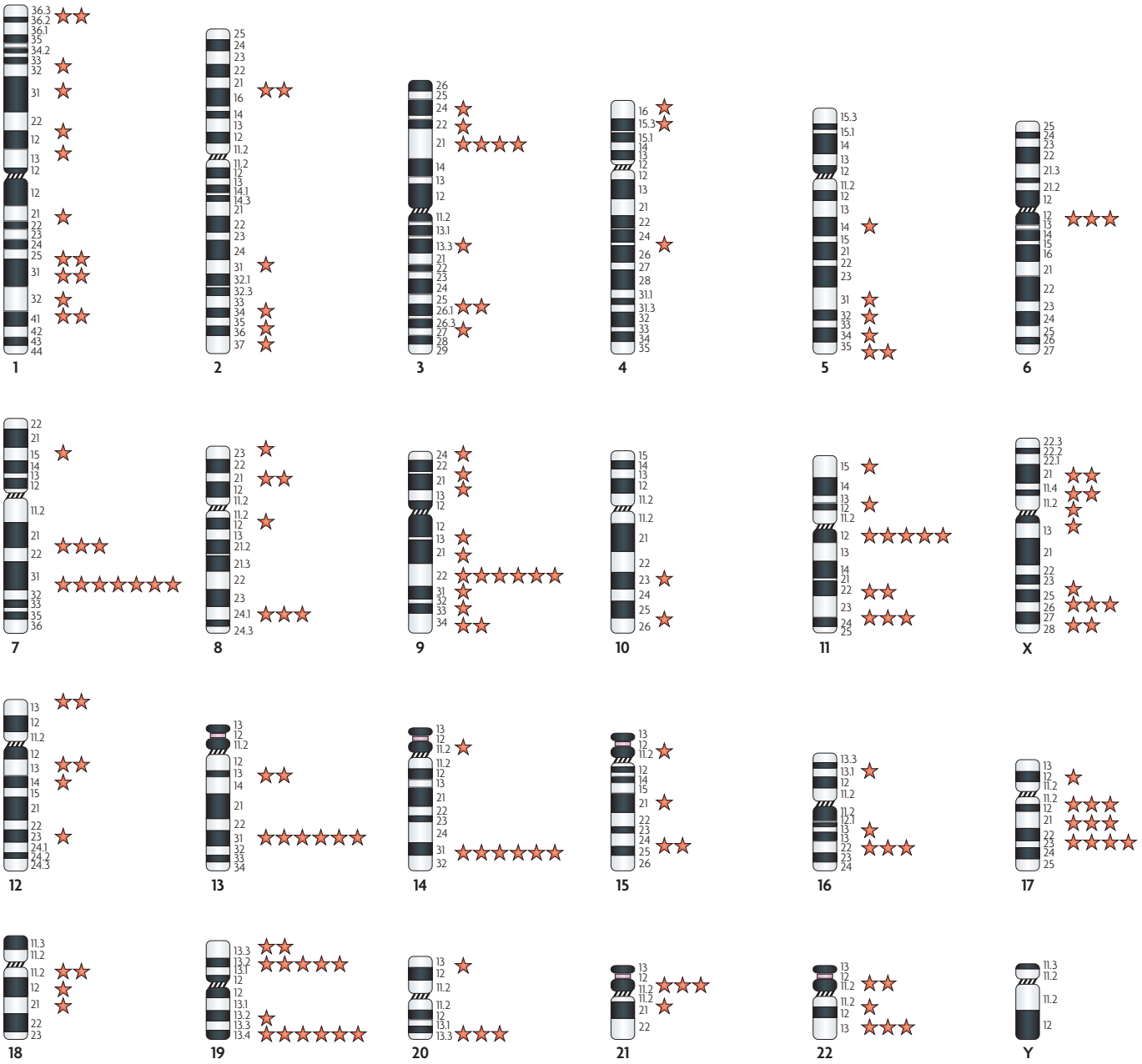

Figure 1 | **MicroRNA genes map to chromosomal regions that are involved in alterations in human cancer.** The 24 human chromosomes are shown, with stars indicating the locations of microRNA-gene-containing regions that are implicated in cancer (one star = one microRNA gene). Many microRNAs map to chromosome regions that are involved in rearrangements in human cancer. For example, *miR-15-a* and *miR-16-1* map to 13q14, a region that is frequently deleted in chronic lymphocytic leukaemia. *miR-29a* and *miR-29b* map to 7q32, a region that is deleted in myelodysplastic syndrome and acute myeloid leukaemia. The *let-7g–let-7a1* cluster maps to 3p2, the *let-7f-1–let-7d* cluster maps to 9q22.3, *let-7a-2* maps to 11q23-q24 and *let-7c* maps to 21q21 — all of these regions are involved in deletions in a range of solid malignancies, including lung, urothelial, breast, ovarian and cervical cancers.

MicroRNAs as tumour suppressors

Every malignancy is heterogeneous because cancer development is the result of a multistep process that involves multiple alterations in oncogenes and tumour suppressor genes over a period of time, often over several years. This heterogeneity is a challenge for understanding the events that initiate cancer development and for identifying potential new therapeutic targets. In terms of therapies, it is important to target the earliest genetic alterations, as these alterations will be present in 100% of the cancer cells, whereas later alterations may be present only in a fraction of the malignant cells. Genetic analysis provides a way to define early targets. Because loss of *miR-15a* and *miR-16-1* was detected in the majority of

indolent CLLs and in aggressive CLLs that originated from the indolent form[15], it seems logical that such genetic alterations must occur quite early during the process of leukaemogenesis. This interpretation was validated by the discovery of mutations that affect the expression of these miRNAs in patients with CLL and by the creation of a mouse CLL model that also carries mutations in the same miRNA locus[20,21]. Further validation is provided by the development of indolent CLL in *miR-15a–miR-16-1* knockout mice (C.M.C., unpublished results).

It is becoming clear that the sequence of genetic events leading to the development of malignancy is not rigid. For example, most indolent CLLs develop a 13q14 deletion that affects the *miR-15a–miR-16-1* locus, and over time these leukaemias might lose miRNA genes at 11q23 or the tumour protein 53 (*TP53*) gene at 17p13, causing them to become aggressive. Aggressive CLLs, however, can start with an 11q23 deletion and then acquire a *miR-15a–miR-16-1* deletion, suggesting that both alterations are necessary for the development of an aggressive CLL. Similarly, in the T cell lymphoma 1 (*TCL1*) transgenic mouse (an aggressive CLL mouse model), dysregulation of *TCL1*, which occurs in all human aggressive CLLs with an 11q23 deletion, is followed by loss of expression of *miR-15a* and *miR-16-1*. The fact that these two different alterations can occur in either order stresses the role of both alterations in the pathogenesis of the aggressive form of CLL. It seems likely that similar situations may occur in other malignancies.

The sequencing of miRNAs that are dysregulated in CLL identified mutations in the precursor of miR-15a and miR-16-1 that affect the processing of the miRNAs in a small fraction of CLL cases[20]. Therefore, not only deletions but also mutations may lead to miRNA loss of function. Interestingly, some of these mutations occurred in the germ line, confirming that the loss of function of *miR-15a* and *miR-16-1* contributes to the development of CLL[20] and indicating that the loss of *miR-15a* and *miR-16-1* expression in the germ line leads to a familial form of CLL. Raveche *et al.* have studied the genetics of an indolent form of CLL that occurs in the NZB mouse strain[21]. This mouse strain develops an indolent form of CLL late in life, as in humans[21]. Having mapped the disease to mouse chromosome 14 in a region homologous to 13q14 in humans, the investigators sequenced the *miR-15a–miR-16-1* locus and found a mutation 6 nucleotides 3′ of *miR-16-1* that also seems to affect processing of the miRNA precursor[21]; in humans, the mutation is 7 nucleotides 3′ of *miR-16-1* (REFS 20,21). Thus, germline mutations that affect the processing of *miR-15a* and *miR-16-1* can cause the indolent form of CLL in both mice and humans. The sequencing of miRNAs in human CLLs also revealed mutations — some germline and some somatic — in other miRNA loci, including *miR-29* family members[20].

Targets of microRNA tumour suppressors

In 2005, Slack and collaborators discovered that *let-7* family members negatively regulate the expression of *RAS*, indicating that the loss of *let-7* family members results in the constitutive overexpression of *RAS*, an oncogene that contributes to the pathogenesis of several types of human tumours[22]. The following year, Cimmino *et al.* discovered that *miR-15a* and *miR-16-1*, which are present in the same polycistronic RNA, target B cell leukaemia/lymphoma 2 (*BCL2*)[23], an oncogene that inhibits apoptosis and is the culprit in another indolent B cell malignancy, follicular lymphoma, due to a consistent t(14;18) chromosomal translocation[24,25]. Therefore, it became clear that the loss of miRNAs could contribute to malignant transformation through the dysfunction of cellular oncogenes, such as *RAS* and *BCL2*. More recently, other targets of these miRNAs have been discovered, providing additional evidence of their role in cancer pathogenesis. For example, *let-7* was also found to target high mobility group AT-hook 2 (*HMGA2*), a gene that is dysregulated in various human tumours, including liposarcoma[26].

It is clear that miRNAs have many different targets — dozens in some cases, hundreds in others — but depending on the cells involved, it seems likely that only a small number of them have a crucial role in cancer pathogenesis. This has been validated by the fact that various alterations in the expression of specific protein targets lead to the same phenotype. In fact, approximately 4% of CLLs carry translocations involving *BCL2* and an immunoglobulin locus that deregulates the oncogene[27,28]. Therefore, overexpression of *BCL2* in CLL seems to be the crucial event in the initiation of most CLLs.

Target-prediction algorithms can be used to identify the protein targets of oncogene overexpression. However, this process must be followed by experimental validation to eliminate false positives, and because the number of predicted targets is usually large, this approach can be laborious. Alternatively, proteomics can be used to identify all of the protein products that are affected by the introduction and expression of a specific miRNA in different cell types[29].

A multitude of microRNA tumour suppressors

Since these early findings, other miRNAs that function as tumour suppressors have been discovered (TABLE 1).

Several of the miRNAs that have been described as suppressors have been found to be deleted or mutated in various human malignancies. For example, loss of *miR-15a* and *miR-16-1* has also been observed in prostate cancer and multiple myeloma (TABLE 1). Members of the *miR-29* family have been found to be deleted in a fraction of myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) patients. Precursors of miR-29 have also been found to be mutated in CLL[20]. Members of the *let-7* family map to regions of the genome that are involved in deletions in several solid and liquid malignancies[16]. In addition, *miR-15a*, *miR-16-1*, *miR-29* family members and *let-7* family members can be silenced by methylation in some cases[12]. In many cases, the involvement of miRNAs as suppressors has been suggested by profiling studies (BOX 1), and alterations in the expression of miRNA genes have been observed in a wide range of tumour types.

Table 1 | **MicroRNAs that function as oncogenes or tumour suppressor genes in human cancers**

MicroRNA	Dysregulation	Function	Validated targets	Oncogene (ONC) or tumour suppressor (TS)	Refs
miR-15a and *miR-16-1*	Loss in CLL, prostate cancer and multiple myeloma	Induces apoptosis and inhibits tumorigenesis	BCL2, WT1 RAB9B and MAGE83	TS	15,20,23, 30,52,69
let-7 (*a*, *b*, *c*, *d*, *e*, *f*, *g* and *i*)	Loss in lung and breast cancer and in various solid and haematopoietic malignancies	Induces apoptosis and inhibits tumorigenesis	RAS, MYC and HMGA2	TS	22,26, 42,70
miR-29 (*a*, *b* and *c*)	Loss in aggressive CLL, AML (11q23), MDS lung and breast cancers and cholangocarcinoma	Induces apoptosis and inhibits tumorigenicity. Reactivates silenced tumour suppressor genes	TCL1, MCL1 and DNMTs	TS	30,64, 71,72
miR-34	Loss in pancreatic, colon, breast and liver cancers	Induces apoptosis	CDK4, CDK6, cyclin E2, EZF3 and MET	TS	56–58
miR-145	Loss in breast cancer	Inhibits proliferation and induces apoptosis of breast cancer cells	ERG	TS	31
miR-221 and *miR-222*	Loss in erythroblastic leukaemia	Inhibits proliferation in erythroblasts	KIT	TS	30
miR-221 and *miR-222*	Overexpression in aggressive CLL, thyroid carcinoma and hepatocellular carcinoma	Promotes cell proliferation and inhibits apoptosis in various solid malignancies	p27, p57, PTEN and TIMP3	ONC	43,51,73
miR-155	Upregulated in aggressive CLL, Burkitt's lymphoma and lung, breast and colon cancers	Induces cell proliferation and leukaemia or lymphoma in mice	MAF and SHIP1	ONC	32–34, 36,37
miR-17–92 cluster	Upregulated in lymphomas and in breast, lung, colon, stomach and pancreatic cancers	Induces proliferation	E2F1, BIM and PTEN	ONC	19,34,35, 40,41
miR-21	Upregulated in glioblastomas, AML (11q23), aggressive CLL and breast, colon, pancreatic, lung, prostate, liver and stomach cancers	Inhibits apoptosis and increases tumorigenicity	PTEN, PDCD4, TPM1 and TIMP3	ONC	31,37–39, 44–50
miR-372 and *miR-373*	Upregulated in testicular tumours	Promotes tumorigenicity in cooperation with RAS	LATS2	ONC	74

AML, acute myeloid leukaemia; BCL2, B cell leukaemia/lymphoma 2; BIM, Bcl2-interacting mediator of cell death; CLL, chronic lymphocytic leukaemia; DNMT, DNA methyltransferase; HMGA2, high mobility group AT-hook 2; LATS2, large tumour suppressor homologue 2; MCL1, myeloid cell leukaemia sequence 1; MDS, myelodysplastic syndrome; PDCD4, programmed cell death 4; PTEN, phosphatase and tensin homologue; SHIP1, SH2 domain-containing inositol-5′-phosphatase 1; TCL1, T cell lymphoma breakpoint 1; TIMP3, tissue inhibitor of metalloproteinases 3; TPM1, tropomyosin 1; WT1, Wilms tumour 1.

Importantly, miRNAs should not be described as oncogenes or tumour suppressor genes, unless the tissue or cell type involved in their action is specified. For example, miR-221 and miR-222 target an oncogene, *KIT*, and inhibit the growth of erythroblastic leukaemia[30], and therefore function as tumour suppressors in erythroblastic cells. But they also target at least four important tumour suppressors — phosphatase and tensin homologue (PTEN), p27, p57 and tissue inhibitor of metalloproteinases 3 (TIMP3) — and function as oncogenic miRNAs by suppressing these tumour suppressors in various human solid tumours[31] (TABLE 1). Therefore, before describing an miRNA as a tumour suppressor or an oncogene, it is necessary to specify in which cell or tissue, as cellular context is crucial for the function of miRNAs[12].

MicroRNAs as oncogenes

The overexpression of miRNAs has been observed in various human tumours, and there is evidence that several of these miRNAs function as oncogenes.

miR-155 *and the* miR-17–92 *cluster.* The first miRNAs that were found to be overexpressed in cancers were miR-155 and the miRNAs encoded by the *miR-17–92* cluster[32-35]. They are amplified at the DNA level in various B cell lymphomas[32,33]. The gene encoding miR-155 is embedded in a host non-protein-coding genomic region named B cell integration cluster and is located on chromosome 21q23 (REF. 32). Several groups have shown that miR-155 is overexpressed in aggressive CLL[20], paediatric Burkitt's lymphoma[36], Hodgkin's lymphoma[37], primary mediastinal non-Hodgkin's lymphoma[37], AML[38], lung cancer[19] and breast cancer[31]. Less is known about the mechanisms of dysregulation of miR-155, with the exception of cases in which the *miR-155* gene is amplified, as in lymphomas.

The *miR-17–92* cluster, which comprises six miRNA genes (*miR-17*, *miR-18a*, *miR-19a*, *miR-20a*, *miR-19b-1* and *miR-92a-1*), is located in a region of 800 bp in the non-protein-coding gene *C13orf25* at 13q31.3 (REF. 34). This chromosomal region was found to be amplified in follicular lymphoma and diffuse large B cell lymphoma[34]. Overexpression of this cluster has been observed in a wide range of human solid tumours, including breast, colon, lung, pancreatic, prostate and stomach cancers, in addition to lymphoma[19]. miRNAs of the *miR-17–92* cluster promote proliferation, inhibit apoptosis, induce tumour angiogenesis and cooperate with *MYC* to accelerate the development of lymphomas[39]. Interestingly, this cluster is transactivated by *MYC*, an oncogene that

is frequently dysregulated in malignancies of various types[35] (see the next section for a more detailed discussion of the cooperation of these miRNAs with *MYC*). Recent studies of gain and loss of function indicated that this cluster is essential for B cell proliferation and that overexpression in B cells induces a lymphoproliferative disorder in mice[40]. This cluster affects cell cycle progression and proliferation through its regulation of the E2F transcription factors[35,41]. Genes of the E2F family encode proteins that activate multiple genes involved in cell cycle progression from G1 to S phase[35,40,41]. It has also been shown that the E2F1 and E2F3 proteins can activate miRNA genes in the *miR-17–92* cluster, which contributes to a regulatory loop[35,41]. miRNAs encoded in the cluster can also downmodulate important targets, such as the tumour suppressors PTEN and p21 and the apoptotic protein Bcl2-interacting mediator of cell death (BIM, also known as Bcl2-like protein 11)[35].

However, experiments aimed at showing the oncogenic potential of the *miR-17–92* cluster have not dissected the role and contribution of each member of the cluster to malignant transformation. Experiments in transgenic mice in which each member of the cluster is dysregulated individually will provide important insights into the role of each of these miRNAs in tumour development. In addition, investigating the processing of each member of the cluster in different cell types may provide interesting novel findings concerning their mechanisms of dysregulation.

The definitive proof that an miRNA can contribute directly to the pathogenesis of a malignancy has been provided by the development of transgenic mice with targeted overexpression of *miR-155* in B cells[33]. Mice that carried a *miR-155* transgene under the control of the immunoglobulin Eμ enhancer expressed high levels of miR-155 in B cells and developed a polyclonal expansion of large pre-B cells followed by frank leukaemia or high-grade lymphoma. Each of these stages was transplantable into syngeneic mice. A few months elapse before the mice develop a frank malignancy, so it is likely that additional genetic alterations occur to convert the pre-malignant cells into malignant cells[33]. These results indicate that the dysregulation of a single oncogenic miRNA can lead to the development of a malignant tumour. Overexpression of miR-155 and loss of let-7 have been found to be poor prognostic indicators for stage 1 lung adenocarcinoma[42].

A multitude of potential oncogenic microRNAs. As indicated in TABLE 1, several additional miRNAs have been found to be overexpressed in various human tumours through profiling and may be candidate oncogenes. The formal proof of this, however, awaits the results of dysregulation experiments in animal models. These overexpressed miRNAs have been found to target important tumour suppressors. For example, miR-21 has been found to target PTEN, programmed cell death 4 (PDCD4), TIMP1 and TIMP3 (REF. 43); miRNAs of the *miR-17–92* cluster have been found to target PTEN, E2F1 and BIM; and miR-221 and miR-222 have been found to target PTEN, p27, p57 and TIMP3 (TABLE 1).

Of particular interest is the fact that *miR-21* was found to be overexpressed in most of the different solid tumours analysed[19]. In fact, overexpression of *miR-21* has been observed in various human tumours, including glioblastoma, breast, lung, colon, pancreatic, liver, stomach and prostate cancers and AML with 11q23 alterations[19,31,38,41,44-50]. In addition, overexpression of miR-21 has been observed during the development and progression of colorectal cancer[50]. Inhibition of miR-21 by antisense oligonucleotides in glioblastoma resulted in cell death[46]. Similarly, inhibition of miR-21 caused apoptosis in liver and breast cancer cells[47]. As mentioned, miR-21 has been found to inhibit several tumour suppressor genes. miR-221 and miR-222 have been found to be overexpressed in many haematopoietic and solid malignancies, such as aggressive CLLs[20], acute leukaemias[38], papillary thyroid carcinoma, hepatocellular carcinoma[51] and colon, pancreatic and prostate cancers[49,50,52].

MicroRNA dysregulation by transcription factors. As mentioned above, the *miR-17–92* cluster, which consists of six miRNAs, is frequently amplified in lymphoma. Members of this cluster have a role in lung development and in the regulation of differentiation in haematopoietic cells. O'Donnell *et al.* have shown that the *miR-17–92* cluster is transactivated by *MYC*, an oncogene that is frequently dysregulated in cancer[53]. Dysregulation of *MYC* by chromosomal translocation and juxtaposition to an immunoglobulin enhancer is the cause of Burkitt's lymphoma[1]. Two recent studies of gain and loss of function showed that this cluster is essential for B cell proliferation and that a modest overexpression in B cells induces a lymphoproliferation disorder in mice[40,41]. This cluster promotes cell cycle progression and proliferation, at least in part through the regulation of E2F transcription factors that regulate cell cycle progression. This cluster also has anti-apoptotic effects through its inhibition of BIM, PTEN and p21 (REF. 54). Members of the *miR-17–92* cluster have homologues in two other clusters. One cluster is on chromosome 7 in the minichromosome maintenance complex component 7 (*MCM7*) gene and consists of *miR-106b*, *miR-93* and *miR-25*, and the other cluster is on the X chromosome and consists of *miR-106a*, *miR-18b*, *miR-20b*, *miR-19b-2*, *miR-92a* and *miR-363*. FIGURE 2 shows the genomic structure of the 3 miRNA clusters on chromosomes 13 and 7 and the X chromosome. *MYC* induces the expression of the cluster on chromosomes 7 and 13 in addition to E2F1, which regulates the *MCM7* and *C13orf25* genes that host the two clusters. At the same time, miR-25 and miR-92a-1 downregulate the pro-apoptotic *BIM* gene, whereas *MYC* induces it[54]. Inactivation of the transforming growth factor-β (TGF-β) tumour suppressor pathway is an important step in the development of a range of human tumours[55]. Two of the three clusters (*miR-106b–25* and *miR-17–92*) function as key modulators of TGF-β signalling in many tumours, interfering with cell cycle arrest and apoptosis when overexpressed in malignant cells[54] (FIG. 2).

Syngeneic mice
Mice that are genetically identical. Cells can be transferred between syngeneic mice without provoking an immune response.

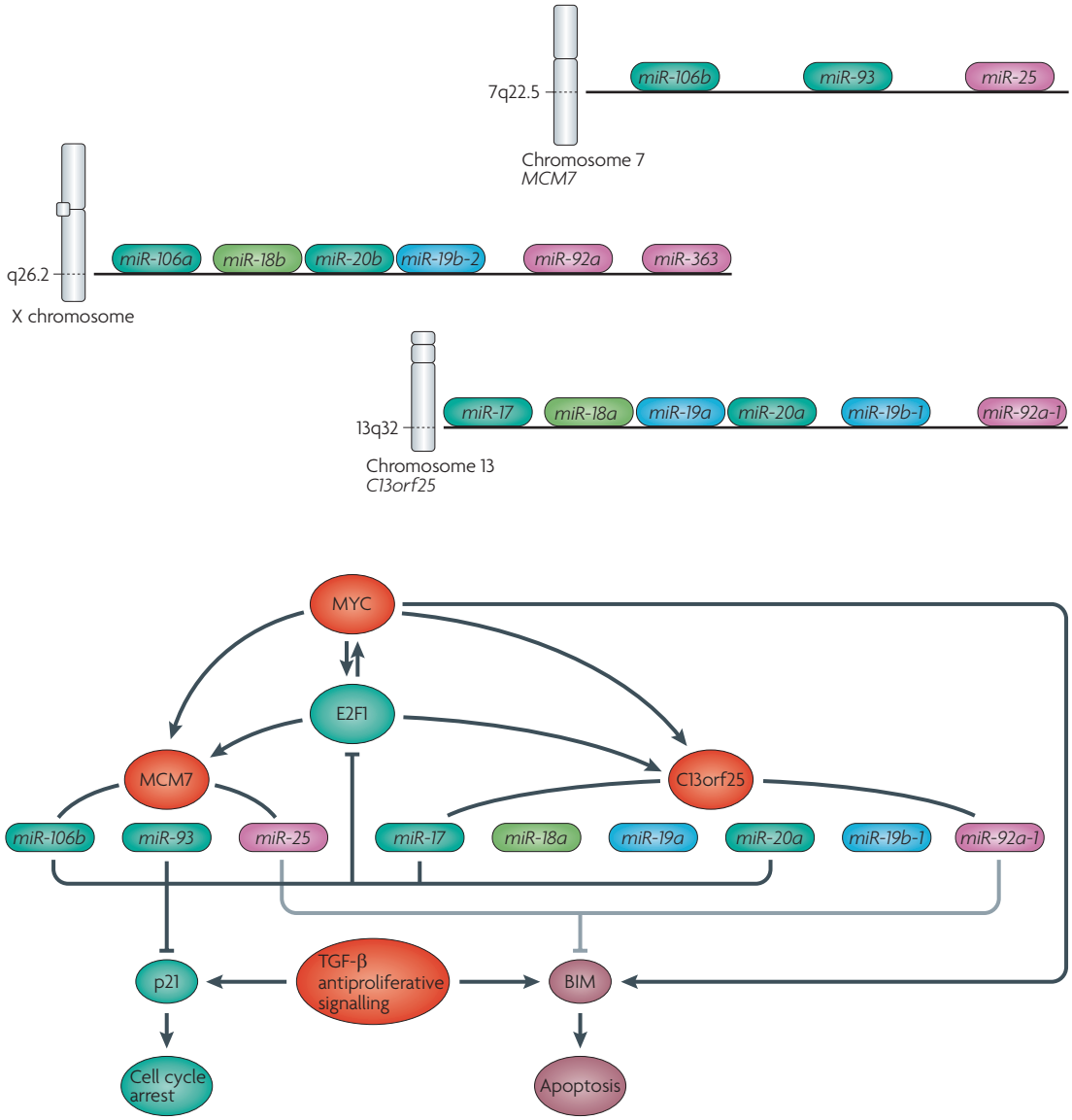

Figure 2 | **Role of the *miR-106b–25* and *miR-17–92* clusters in the control of transforming growth factor-β signalling.** This figure shows the genomic organization of three related microRNA (miRNA) clusters that map to chromosomes 13 and 7 and the X chromosome. Homology between the different individual miRNAs is indicated by the same colours. *miR-106b–25* and *miR-17–92* regulate the MYC–E2F1–transforming growth factor-β (TGF-β) network. The minichromosome maintenance complex component 7 (*MCM7*) and *C13orf25* genes, which host the miRNA clusters, are induced by MYC and E2F1. Increased expression of the *miR-106* family, which consists of *miR-106b*, *miR-93*, *miR-17* and *miR-20a*, causes reduced expression of p21, a cell cycle inhibitor, therefore inhibiting TGF-β-dependent cell cycle arrest. Increased expression of *miR-25* and *miR-92a-1* affects TGF-β-induced apoptosis through the inhibition of Bcl2-interacting mediator of cell death (BIM) expression. Because overexpression of MYC induces apoptosis, possibly through the induction of BIM, increased expression of *miR-25* and *miR-92a-1* can prevent MYC-induced apoptosis in cancer cells.

It seems likely that normally these two clusters dictate the pace of the TGF-β response by repressing BIM and p21 expression in proliferating cells and allowing sequential expression of p21 and BIM as cells differentiate and undergo TGF-β-induced cell cycle arrest and apoptosis. The work of Ventura *et al.* shows that the two clusters control apoptosis, as *miR-17–92* and *miR-106b–25* double knockout mice have a much more severe phenotype compared with *miR-17–92* single knockout mice[41]. In cancer development, dysregulation of *miR-106b–25* and/or *miR-17–92* would compromise this regulatory mechanism, providing an escape from TGF-β-induced cell cycle arrest and apoptosis.

Recently, several reports indicated that the *miR-34* family (which consists of *miR-34a*, *miR-34b* and *miR-34c*) is induced directly by the p53 tumour suppressor[56–58] and suggested that some p53 effects could be mediated by these miRNAs. By using various cellular models, the authors compared miRNA expression in cells with high or low levels of p53 expression and found that

Table 2 | **Consequences of microRNA dysregulation in human cancers**

MicroRNA dysregulation	Targets	Consequences
MicroRNA overexpression	Tumour suppressors	Downregulation of tumour suppressors — for example, PTEN, p22, p57, TIMP3 and PDCD4
MicroRNA loss	Oncogenes	Upregulation of oncogenes — for example, *BCL2*, *MCL1*, *RAS*, *HMGA2*, *MYC* and *MET*
MicroRNA loss	DNA methyltransferases	Downregulation of tumour suppressors — for example, p16, FHIT and WWOX
MicroRNA loss	Chromatin silencers	Downregulation of tumour suppressors

BCL2, B cell leukaemia/lymphoma 2; FHIT, fragile histidine triad protein; *HMGA2*, high mobility group AT-hook 2; *MCL1*, myeloid cell leukaemia sequence 1; PDCD4, programmed cell death 4; PTEN, phosphatase and tensin homologue; TIMP3, tissue inhibitor of metalloproteinases 3; WWOX, WW domain-containing oxidoreductase.

the expression levels of p53 correlated with the levels of expression of *miR-34* family members. In addition, chromatin immunoprecipitation experiments showed that p53 binds to the *miR-34* promoters[57,58]. The introduction of *miR-34* family members into cells that had lost *miR-34* expression resulted in cell cycle arrest. To assess the role of each *miR-34* family member, it will be necessary to knock out the expression of each member in different mouse tissues to determine whether loss of expression leads to malignant transformation or to a higher susceptibility to a range of tumours.

Breast cancer is the second leading cause of death among women in the Western world and its molecular pathogenesis is still not completely understood. Several lines of evidence indicate that oestrogen receptor-α-negative ($ERα^-$) breast tumours, which are highly aggressive and unresponsive to hormonal therapy, arise from ERα-positive ($ERα^+$) precursors through different molecular pathways. High levels of miR-221 and miR-222 were found in $ERα^-$ cells and breast tumour samples. Overexpression of miR-221 and miR-222 in $ERα^+$ cells suppressed ERα protein and luciferase assays, and confirmed that ERα is a target of miR-221 and miR-222. ERα was also found to negatively regulate expression of miR-221 and miR-222 by promoter binding[59]. Therefore, silencing ERα — for example, by methylation or by dysregulating miR-221 and miR-222 through the activation of pathways, such as the MET pathway, that are involved in oncogenesis — results in the constitutive activation of miR-221 and miR-222 and inhibition of the tumour suppressors p27, p57, PTEN and TIMP3, which contributes to the development of the invasive phenotype that is characteristic of frankly malignant cells[43]. This regulatory feedback loop seems to be involved in the development of $ERα^-$ breast cancers[55].

So, miRNAs can be dysregulated by transcription factors and, therefore, genetic or epigenetic alterations that result in the dysregulation of transcription factors can cause miRNA dysregulation, which contributes to malignant transformation (TABLE 2). Volinia *et al.* have defined miRNA expression signatures that distinguish cancerous tissues from normal tissues[19]. Interestingly, they observed that some miRNA genes were dysregulated not just in one tumour type but in many, suggesting that these miRNAs may be downstream targets of pathways that are commonly dysregulated in cancer[19].

If miRNAs are downstream targets of pathways that are commonly dysregulated in human cancer (BOX 1), they become excellent targets for therapeutic intervention. It is known that proliferative signals lead to the activation of the MYC transcription factor, which positively and negatively regulates the expression of many protein-coding genes and miRNA genes, including the *miR-17–92* cluster (which it regulates positively) and *miR-15a* and *miR-16-1* (which it regulates negatively). Therefore, it seems possible that the mechanism of action of the activated oncogene is, at least partly, miRNA dysregulation — overexpression of miR-17–92 causes the downregulation of tumour suppressors and underexpression of miR-15a and miR-16-1 causes the upregulation of oncogenes, such as *BCL2* and myeloid cell leukaemia sequence 1 (*MCL1*). Therefore, genetic or epigenetic alterations in protein-coding cancer genes or in miRNA genes may have similar consequences. Until now it has not been possible to target the overexpression of *MYC* in tumours with drugs, but it is possible to target the miRNAs that are dysregulated by *MYC* — for example, by treating the cancer cells with anti-miR-17–92 or with miR-15a and miR-16-1. Recently, Bonci *et al.* have shown that the *miR-15a–miR-16-1* cluster can control prostate cancer by targeting multiple oncogenic activities[60]. More generally, in tumours with alterations in protein-coding cancer genes, it should be possible to induce tumour regression using miRNAs and anti-miRNAs. In fact, recently Kota *et al.* have regressed Myc-induced hepatocellular carcinomas in mice by targeting an miRNA that is induced by Myc[61].

Roles of microRNAs in cancer epigenetics

Altered patterns of epigenetic modifications, in particular the methylation of CpG islands in the promoter regions of tumour suppressors is a common finding in many tumours (for a review, see REF. 62). Such changes are often accompanied by aberrant patterns of histone modification. Therefore, the silencing of tumour suppressors in cancer can be caused not only by deletions and mutations but also by epigenetic changes.

MicroRNAs as targets of epigenetic changes. The most studied epigenetic changes in cancer cells are the methylation of cytosines in the dinucleotide CpG in DNA[62]. Such 'methylable' sites, known as CpG islands, are preferentially located in the 5′ region (which consists of

the promoter, 5′ UTR and exon 1) of many genes, are non-methylated in normal cells and are transcribed in the presence of the appropriate transcription factors. Methylation of the CpG islands of tumour suppressors results in their silencing and contributes to malignant transformation[62]. As mentioned above, the expression of miRNAs can be affected by genetic changes, such as deletion, gene amplification and mutation, and by transcription factors. In addition, the expression of miRNAs can be affected by epigenetic changes, such as methylation of the CpG islands of their promoters. Saito *et al.* reported that *miR-127* is silenced by promoter methylation in bladder tumours and that its expression could be restored by using hypomethylating agents such as azacitidine[63]. This miRNA targets *BCL6*, an oncogene that is involved in the development of diffuse large B cell lymphoma. Therefore, the silencing of *miR-127* may lead to the overexpression of BCL6 (REF. 63). Other investigators have described additional miRNAs that are silenced by methylation in various cancers and that can be reactivated by hypomethylating agents.

MicroRNAs as regulators of epigenetic changes. miRNAs can also regulate enzymes that are involved in the methylation of the CpG islands of tumour suppressor genes. For example, the overexpression of DNA methyltransferases (DNMTs) is a poor prognostic indicator, and miRNAs of the miR-29 family target *de novo* DNMT3A and DNMT3B [64]. Interestingly, the introduction of miR-29 family members into lung cancer cell lines caused demethylation of the CpG islands in the promoter regions of tumour suppressor genes, which allowed their reactivation and resulted in the loss of tumorigenicity[64].

Similarly, a recent study by Garzon *et al.* has shown that miR-29s target not only DNMT3A and DNMT3B but also, indirectly, the DNMT1 maintenance DNMT[65]. The introduction of miR-29 into AML cells resulted in the loss of expression of the oncogene *MCL1* and the three DNMTs, in addition to the reactivation of the p16 tumour suppressor[65]; therefore, treatment of the malignancies with miR-29 family members caused the reversion of epigenetic changes that contribute to malignant transformation in human cancers, such as lung cancer and AML.

Garzon *et al.*[66] have also shown that expression of the *miR-29* cluster encoded at chromosome 7q32 is downregulated in acute leukaemias with deletions of 7q, an alteration that is frequently observed in MDS and AML. Therefore, miR-29s could be considered as candidate drugs for the treatment of these malignancies. Because loss of miR-29s is observed in patients with MDS and AML, an alternative therapeutic strategy could be to stratify patients on the basis of miR-29 expression and treat them with azacitidine or decitabine, which would lead to demethylation and the reactivation of tumour suppressors. The prediction is that only those that have lost miR-29 expression will respond. Other miRNAs are predicted to target the DNMTs that are responsible for the methylation of the CpG islands of tumour suppressors. We already have preliminary evidence that miR-148a and miR-148b, which are lost in a range of human tumours, also target DNMTs (C.M.C., unpublished results). miRNAs are also predicted to target HDACs and other proteins that are involved in chromatin structure and function[67]; therefore, it will not be surprising if the dysregulation of these miRNAs contributes to cancer pathogenesis.

These observations indicate that alterations in the expression of miRNAs could be responsible for some of the epigenetic changes that are observed in cancer cells, and that such miRNAs could provide novel targets for cancer therapy (TABLE 2).

Therapies based on microRNAs or anti-microRNAs

The studies described in the sections above have highlighted a range of examples in which miRNA genes are involved or implicated in the pathogenesis of human cancer. The fact that several miRNA genes are dysregulated in multiple types of cancer also suggests that crucial pathways that are involved consistently in tumorigenesis may have miRNA genes as downstream targets[19]. Therefore, in tumours in which miRNAs are lost or overexpressed, miRNAs or anti-miRNAs[68], respectively, could be considered as drugs that induce apoptosis and/or cell cycle arrest in cancer cells that depend on miRNA dysregulation for growth and survival.

The introduction of miRNAs or anti-miRNAs into tumours can be achieved in many different ways. They can be introduced into vectors, such as adenoviruses, adeno-associated viruses (AAVs) or retroviruses, which can deliver the miRNA to the tissue of interest[61]. Alternatively, miRNAs or anti-miRNAs can be used directly without the use of an expression vector; these miRNAs or anti-miRNAs can be modified to increase their half-life *in vivo* or be used in an unmodified form. The issue of delivery is important for therapeutics. Some tissues — for example, liver, bone marrow, spleen and kidney — will readily take up miRNAs. In these cases it may not be necessary to use vectors or modified miRNAs and anti-miRNAs. In other cases in which the natural uptake is modest, it may be necessary to use vectors or other delivery systems, such as liposomes.

Experiments in mice indicate that miRNAs and anti-miRNAs could be used for therapy. For example, miR-15a and miR-16-1 have been used to suppress the growth of leukaemic cells that lack these miRNAs[29], and miR-29 family members have been used to suppress the tumorigenicity of lung cancer cells[64]. Members of the miR-29 family have also been used to suppress the growth of human AML cells that lack these miRNAs and to stem the overexpression of MCL1 and DNMTs in mice[66]. Therefore, it is likely that targeted therapies that exploit miRNA dysregulation in cancer can be developed for the treatment of human malignancies. In addition, therapeutic experiments using miRNAs or anti-miRNAs in mice have suggested that, like imatinib treatment of CML patients, these treatments have modest side effects, if any. miRNA profiling could also be used for patient stratification and for treatment selection, as miRNAs have also been found to cause drug resistance. It is interesting that some, perhaps many, of the epigenetic

Liposome
An artificial lipid vesicle. Liposomes fuse with the cell membrane to deliver their contents, such as DNA for gene therapy.

changes observed in cancer may be due to miRNA dysregulation. Therefore, miRNAs could be used to reverse these changes. In fact, as mentioned, the loss of miR-29 family members was discovered in lung cancer, AML and aggressive CLL. Such miRNAs have been found to target DNMTs and reactivate silenced tumour suppressor genes[64,66]. DNMTs are also targeted by other miRNAs that are lost in human tumours. HDACs and other proteins that are involved in chromatin structure and function are also predicted to be regulated by miRNAs[67]. Therefore, it is not far-fetched to imagine that miRNAs will be used for reversing changes in chromatin structure that are associated with tumorigenesis. Furthermore, knowing which miRNAs are involved in directing the epigenetic marks that are lost in cancer will allow better patient stratification and the use of currently available drugs to revert those changes. For example, because miR-29s are lost in AML in which 7q is deleted and because AML cells with 7q deletions *in vitro* can be induced to re-express silenced tumour suppressor genes with miR-29, it should be possible to stratify AML patients on the basis of miR-29 expression to determine whether only those with loss of miR-29 respond to the methylation inhibitors azacitidine and decitabine.

Conclusions and future challenges

Since the discovery of *miR-15a* and *miR-16-1* deletions in CLL[15], many laboratories around the world have shown miRNA dysregulation in all tumours studied, including the most common, such as lung, breast, prostate and gastrointestinal cancers. Such dysregulation, like the dysregulation of oncogenes and tumour suppressor genes, can be caused by multiple mechanisms, such as deletion, amplification, mutation, transcriptional dysregulation and epigenetic changes. As miRNAs have multiple targets, their function in tumorigenesis could be due to their regulation of a few specific targets, possibly even one, or many targets. A future challenge will be to identify all of the targets of the miRNAs involved in cancer and establish their contribution to malignant transformation. An additional challenge will be the identification of all of the miRNAs that are dysregulated by pathways that are consistently dysregulated in various types of human cancers. This point is of particular importance, as instead of focusing on specific alterations in protein-coding oncogenes or tumour suppressor genes — which may be difficult to treat — we could focus on their downstream miRNA targets. If these miRNA targets are crucial for the expression of the malignant phenotype and the cancer cells depend on their dysregulation for proliferation and survival, we can expect that the use of miRNAs or anti-miRNAs will result in tumour regression. Genomic analyses for alteration in miRNA genes or for copy number alterations in various human tumours by deep sequencing is in progress but has not been completed. These studies could provide additional information concerning the involvements of miRNAs in cancer and in many other diseases. Over the past few years, we have observed a shift from conventional chemotherapy to targeted therapies, and miRNAs and anti-miRNAs will contribute extensively to the latter.

1. Croce, C. M. Oncogenes and cancer. *N. Engl. J. Med.* **358**, 502–511 (2008).
2. Groffen, J. *et al.* Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell* **36**, 93–99 (1984).
3. Shtivelman, E., Lifshitz, B., Gale, R. P. & Canaani, E. Fused transcript of *abl* and *bcr* genes in chronic myelogenous leukaemia. *Nature* **315**, 550–554 (1985).
4. Druker, B. J. *et al.* Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* **344**, 1031–1037 (2001).
5. Roche-Lestienne, C. *et al.* Several types of mutations of the *Abl* gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood* **100**, 1014–1018 (2002).
6. Jones, P. A. & Baylin, S. B. The epigenomics of cancer. *Cell* **128**, 683–692 (2007).
7. Okano, M., Bell, D. W., Haber, D. A. & Li, E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell* **99**, 247–257 (1999).
8. Bots, M. & Johnstone, R. W. Rational combinations using HDAC inhibitors. *Clin. Cancer Res.* **15**, 3970–3977 (2009).
9. Lee, R. C., Feinbaum, R. L. & Ambros, V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with anti-sense complementarity to *lin-14*. *Cell* **75**, 843–854 (1993).
This paper describes the discovery of the first miRNA gene.
10. Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism and function. *Cell* **116**, 281–297 (2004).
11. Esquela-Kerscher, A. & Slack, F. J. Oncomirs — microRNAs with a role in cancer. *Nature Rev. Cancer* **6**, 259–269 (2006).
An excellent review on the role of miRNAs in human malignancies.
12. Calin, G. A. & Croce, C. M. MicroRNA signatures in human cancers. *Nature Rev. Cancer* **6**, 857–866 (2006).
13. Lagos-Quintana, M., Rauhut, R., Lendeckel, W. & Tuschl, T. Identification of novel genes coding for small expressed RNAs. *Science* **294**, 853–858 (2001).
14. Bullrich, F. *et al.* Characterization of the 13q14 tumor suppressor locus in CLL: identification of *ALT1*, an alternative splice variant of the *LEU2* gene. *Cancer Res.* **61**, 6640–6648 (2001).
15. Calin, G. A. *et al.* Frequent deletions and down-regulation of micro-RNA genes *miR15* and *miR16* at 13q14 in chronic lymphocytic leukemia. *Proc. Natl Acad. Sci. USA* **99**, 15524–15529 (2002).
The first evidence of alterations of miRNA genes and dysregulation of miRNA gene expression in human disease.
16. Calin, G. A. *et al.* Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc. Natl Acad. Sci. USA* **101**, 2999–3004 (2004).
The first evidence that many miRNA genes are located in genomic regions that are altered in human cancer.
17. Esquela-Kerscher, A. *et al.* The *let-7* microRNA reduces tumor growth in mouse models of lung cancer. *Cell Cycle* **7**, 759–764 (2008).
18. Chin, L. J. *et al.* A SNP in a *let-7* microRNA complementary site in the *KRAS* 3′ untranslated region increases non-small cell lung cancer risk. *Cancer Res.* **68**, 8535–8540 (2008).
19. Volinia, S. *et al.* MicroRNA expression signature of human solid tumors defines cancer gene targets. *Proc. Natl Acad. Sci. USA* **103**, 2257–2261 (2006).
20. Calin, G. A. *et al.* A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N. Engl. J. Med.* **353**, 1793–1801 (2005).
The first evidence of somatic and germline mutations in miRNA genes in disease.
21. Raveche, E. S. *et al.* Abnormal microRNA-16 locus with synteny to human 13q14 linked to CLL in NZB mice. *Blood* **109**, 5079–5086 (2007).
The first evidence of a germline mutation in an miRNA precursor that predisposes to a malignancy in mice.
22. Johnson, S. M. *et al. RAS* is regulated by the *let-7* microRNA family. *Cell* **120**, 635–647 (2005).
The first evidence of an miRNA that targets an oncogene.
23. Cimmino, A. *et al. miR-15* and *miR-16* induce apoptosis by targeting BCL2. *Proc. Natl Acad. Sci. USA* **102**, 13944–13949 (2006).
The first evidence that miRNAs target an oncogene that is involved in the inhibition of apoptosis.
24. Tsujimoto, Y. *et al.* Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science* **226**, 1097–1099 (1984).
25. Tsujimoto, Y., Cossman, J., Jaffe, E. & Croce, C. M. Involvement of the bcl-2 gene in human follicular lymphoma. *Science* **228**, 1440–1443 (1985).
26. Lee, Y. A. & Dutta, A. The tumor suppressor microRNA *let-7* represses the HMGA2 oncogene. *Genes Develop.* **21**, 1025–1030 (2007).
27. Adachi, M., Cossman, J., Longo, D., Croce, C. M. & Tsujimoto, Y. Variant translocation of the *bcl-2* gene to immunoglobulin λ light chain gene in chronic lymphocytic leukemia. *Proc. Natl Acad. Sci. USA* **86**, 2771–2774 (1989).
28. Adachi, M., Tefferi, A., Greipp, P. R., Kipps, T. J. & Tsujimoto, Y. Preferential linkage of bcl-2 to immunoglobulin light chain gene in chronic lymphocytic leukemia. *J. Exp. Med.* **171**, 559–564 (1990).
29. Calin, G. A. *et al.* MiR-15a and miR-16-1 cluster functions in human leukemia. *Proc. Natl Acad. Sci. USA* **105**, 5166–5171 (2008).
30. Felli, N. *et al.* MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down-modulation. *Proc. Natl Acad. Sci. USA* **102**, 18081–18086 (2005).
31. Iorio, M. V. *et al.* MicroRNA gene expression deregulation in human breast cancer. *Cancer Res.* **65**, 7065–7070 (2005).
32. Eis, P. S. *et al.* Accumulation of miR-155 and *BIC* RNA in human B cell lymphomas. *Proc. Natl Acad. Sci. USA* **102**, 3627–3632 (2005).

33. Costinean, S. *et al.* Pre-B cell proliferation and lymphoblastic leukemia/high grade lymphoma in Eμ-miR155 transgenic mice. *Proc. Natl Acad. Sci. USA* **103**, 7024–7029 (2006).
This paper provides the first direct evidence that the dysregulation of a single miRNA can lead to cancer.
34. Ota, A. *et al.* Identification and characterization of a novel gene, *C13orf25*, as a target for 13q31–q32 amplification in malignant lymphoma. *Cancer Res.* **64**, 3087–3095 (2004).
35. Mendell, J. T. miRiad roles for the miR-17-92 cluster in development and disease. *Cell* **133**, 217–222 (2008).
36. Metzler, M., Wilda, M., Busch, K., Viehmann, S. & Borkhardt, A. High expression of precursor microRNA-155/*BIC* RNA in children with Burkitt lymphoma. *Genes Chromosom. Cancer* **39**, 167–169 (2004).
37. Kluiver, J. *et al.* BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. *J. Pathol.* **207**, 243–249 (2005).
38. Garzon, R. *et al.* MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. *Blood* **111**, 3183–3189 (2008).
39. He, L. *et al.* A microRNA polycistron as a potential human oncogene. *Nature* **435**, 828–833 (2005).
40. Xiao, C. *et al.* Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. *Nature Immunol.* **9**, 405–414 (2008).
41. Ventura, A. G. *et al.* Targeted deletion reveals essential and overlapping functions of the *miR-17 ~ 92* family of miRNA clusters. *Cell* **132**, 875–886 (2008).
42. Yanaihara, N. *et al.* Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* **9**, 189–198 (2006).
43. Garofalo, M. *et al.* MiR-221 and 222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. *Cancer Cell* (in the press).
44. Ciafrè, S. A. *et al.* Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem. Biophys. Res. Commun.* **334**, 1351–1358 (2005).
45. Meng, F. *et al.* MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* **133**, 647–658 (2007).
46. Chan, J. A., Kritchevsky, A. M. & Kasic, K. S. MicroRNA-21 is an anti-apoptotic factor in human glioblastoma cells. *Cancer Res.* **65**, 6029–6033 (2005).
47. Frankel, L. B. *et al.* Programmed cell death 4 (PDCD4) is an important functional target of the microRNA *miR-21* in breast cancer cells. *J. Biol. Chem.* **283**, 1026–1033 (2008).
48. Zhu, S., Si, M.-L., Win, H. & Mo, Y.-Y. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (*TPM1*). *J. Biol. Chem.* **282**, 14328–14336 (2007).
49. Bloomston, M. *et al.* MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA* **297**, 1901–1908 (2007).
50. Schetter, A. J. *et al.* MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA* **299**, 425–436 (2008).
51. Fornari, F. *et al.* MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. *Oncogene* **27**, 5651–5661 (2008).
52. Ambs, S. *et al.* Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. *Cancer Res.* **68**, 6162–6170 (2008).
53. O'Donnell, K. A. *et al.* c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* **435**, 839–843 (2005).
54. Petrocca, F. *et al.* E2F1-regulated microRNAs impair TGFβ-dependent cell-cycle arrest and apoptosis in gastric cancer. *Cancer Cell* **13**, 272–286 (2008).
55. Derynck, R., Akhurst, R. J. & Balmain, A. TGF-β signaling in tumor suppression and cancer progression. *Nature Genet.* **29**, 117–129 (2001).
56. He, L. *et al.* A microRNA component of the p53 tumour suppressor network. *Nature* **447**, 1130–1134 (2007).
57. Raver-Shapira, N. *et al.* Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol. Cell* **26**, 731–743 (2007).
58. Chang, T.-C. *et al.* Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol. Cell* **26**, 745–752 (2007).
59. DiLeva, G. *et al.* A regulatory 'miRcircuitry' inducing miR-221 and 222 and ERα determines ERα status of breast cancer cells. *J. Natl Cancer Inst.* (in the press).
60. Bonci, D. *et al.* The *miR-15a–miR-16-1* cluster controls prostate cancer by targeting multiple oncogenic activities. *Nature Med.* **14**, 1271–1277 (2008).
61. Kota, J. *et al.* Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* **137**, 1005–1017 (2009).
62. Esteller, M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nature Rev. Genet.* **8**, 286–298 (2007).
63. Saito, Y. *et al.* Specific activation of microRNA-127 with downregulation of the proto-oncogene *BCL6* by chromatin-modifying drugs in human cancer cells. *Cancer Cell* **9**, 435–443 (2006).
64. Fabbri, M. *et al.* MicroRNA family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc. Natl Acad. Sci. USA* **104**, 15805–15810 (2007).
The first evidence that miRNAs can affect epigenetic changes and cause the reactivation of silenced tumour suppressor genes.
65. Garzon, R. *et al.* MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly *DNMT3A* and *3B* and indirectly *DNMT1*. *Blood* **113**, 6411–6418 (2009).
66. Garzon, R. *et al.* MicroRNA-29 expression and function in acute myeloid leukemia. *Blood* (in the press).
67. Noonan, E. J. *et al.* miR-449a targets HDAC-1 and induces growth arrest in prostate cancer. *Oncogene* **28**, 1714–1724 (2009).
68. Krutzfeldt, J. *et al.* Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature* **438**, 685–689 (2005).
69. Roccaro, A. M. *et al.* MicroRNAs 15a and 16 regulate tumor proliferation in multiple myeloma. *Blood* **26**, 6669–6680 (2009).
70. Sampson, V. B. *et al.* MicroRNA let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. *Cancer Res.* **67**, 9762–9770 (2007).
71. Mott, J. L., Kobayashi, S., Bronk, S. F. & Gores, G. J. miR-29 regulates Mcl-1 protein expression and apoptosis. *Oncogene* **26**, 6133–6140 (2007).
72. Pekarsky, Y. *et al.* Tcl1 expression in chronic lymphocytic leukemia is regulated by *miR-29* and *miR-181*. *Cancer Res.* **66**, 11590–11593 (2006).
73. le Sage, C. *et al.* Regulation of the $p27^{Kip1}$ tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *EMBO J.* **26**, 3699–3708 (2007).
74. Voorhoeve, P. M. *et al.* A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell* **124**, 1169–1181 (2006).

DATABASES

miRBase: http://microrna.sanger.ac.uk/sequences
lin-4 | miR-155 | *miR-15a* | *miR-15b* | *miR-16-1* | *miR-16-2* | *miR-17* | *miR-18a* | *miR-19a* | *miR-19b-1* | *miR-20a* | miR-221 | miR-222 | *miR-34a* | *miR-34b* | *miR-34c* | *miR-92a-1*

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