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miRNAs and Cancer: an Epigenetics View

Marcos Malumbres*

Cell Division and Cancer Group, Spanish National Cancer Research Centre (CNIO), Melchor Fernández Almagro 3, E-28029, Madrid, Spain

Abstract

microRNAs (miRNAs) are small, non-coding RNAs with critical roles in fine-tuning a wide array of biological processes including development, metabolism, and homeostasis. miRNAs expression, similarly to that of protein-coding genes, is regulated by multiple transcriptional networks as well as the epigenetic machinery. miRNA genes can be epigenetically regulated by DNA methylation or specific histone modifications. In addition, miRNAs can themselves repress key enzymes that drive epigenetic remodeling, generating regulatory circuits that have a significant effect in the transcriptional landscape of the cell. Recent evidences also suggest that miRNAs can directly modulate gene transcription in the nucleus through the recognition of specific target sites in promoter regions. Given the widespread distribution of epigenetic marks and miRNA target sites in the genome, the regulatory circuits linking both mechanisms are likely to have a major impact in genome transcription and cell physiology. Not surprisingly, tumor-associated aberrations in the miRNA or epigenetic machineries are widely distributed in human cancer, and we are just starting to understand their relevance in diagnosis, prognosis or therapy.

Keywords

Cell proliferation; Epigenetics; Metastasis; microRNAs; Transcriptional gene silencing; Tumor development

1 Introduction

Non-protein-coding RNAs are emerging as a new class of regulatory genes with critical relevance in all cellular processes. microRNAs (miRNAs) are small, single-stranded, non-coding RNAs (19-25 nucleotides in length) that are generated from hairpin transcripts (Ambros, 2004; Kim, 2005). miRNA genes are usually transcribed by RNA polymerase II, similarly to most genes in the genome, generating long primary transcripts (pri-miRNAs; Fig. 1). These primary transcripts are processed by Drosha (a RNase type III) complexes containing the co-factor Di George syndrome critical region 8 (DGCR8) giving rise to 70-100 nt long pre-miRNAs. An alternative, Drosha-independent, pathway that depends on the splicing machinery has also been described in several species (Yang and Lai, 2011). pre-miRNAs are then processed by the RNase III Dicer to generate a double-stranded (ds)RNA approximately 22 nt long. This dsRNA includes the mature miRNA (also known as

*Corresponding author. M. Malumbres, Spanish National Cancer Research Centre (CNIO), Melchor Fernández Almagro 3, E-28029, Madrid, Spain. Tel. +34 91 7328000; Fax +34 91 /328033; malumbres@cnio.es.

miRNA-5p; in red in Fig. 1) and the complementary strand known as miRNA* or miRNA-3p. miRNAs* are normally subjected to degradation although they may also act as functional microRNAs. The mature single-stranded microRNA is then able to modulate the expression of target protein-coding mRNAs by base-pairing to partially complementary regions frequently located at the 3'-untranslated regions (3'-UTR) of the target transcript (Fig. 1). The mature miRNA recognizes specific sequences in the mRNAs and recruits Argonaute (Ago) proteins as well as other additional factors such as the trans-activator RNA binding protein (TRBP) in a complex known as RISC (RNA-induced silencing complex). RISC is responsible for site-specific cleavage of the mRNA, enhanced mRNA degradation and repression of its translation (Ambros, 2004; Kim, 2005; Yang and Lai, 2011). Recent evidences suggest that miRNAs can also act on transcripts in a RISC-independent manner (Eiring et al., 2010).

More than a thousand miRNAs exist in the human genome and they are able to modulate the expression of 30-60% of the protein coding genes. In addition, most transcripts can be modulated by multiple miRNAs. Through this regulation, miRNAs play critical roles in development, differentiation, cell proliferation, apoptosis and stress responses. Not surprisingly, these small non-coding RNAs are also implicated in tumor development by regulating the cellular levels of specific oncogenes or tumor suppressor genes (Iorio and Croce, 2012; Lee and Dutta, 2009). The expression of miRNAs is altered in tumor cells by genetic or epigenetic mechanisms or by aberrant expression of transcription factors. In addition, the activity of some miRNAs can also modulate the epigenetic regulation of multiple genes by controlling the levels of DNA methyltransferases or histone deacetylases, among other epigenetic regulators (Iorio and Croce, 2012; Iorio et al., 2010; Kunej et al., 2011; Sato et al., 2011). Finally, recent evidences suggest that miRNAs are able to bind complementary sequences in gene promoters, recruiting specific protein complexes that modulate chromatin structure and gene expression (Fig. 1). The relevant role of miRNAs in tumor development has prompted the search and evaluation of therapeutic strategies targeting these small sequences for cancer therapy (Garofalo and Croce, 2011; Garzon et al., 2010; Iorio and Croce, 2012; Trang et al., 2008).

2 miRNAs and tumor development

miRNAs were initially discovered in 1993 when a small RNA (lin-4) was associated to the development of the nematode *Caenorhabditis elegans* (Lee et al., 1993). Several small regulatory RNAs were identified in plants and animals and designated "microRNAs" in 2001 (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001). These pioneer studies suggested that these small sequences could modulate the expression of critical genes involved in development. Their role in human cancer was not clearly established until the group of C.M. Croce found consistent genetic alteration of a specific miRNA cluster in chronic lymphocytic leukemia (CLL). The miRNA gene encoding miR-15a and miR-16-1, at human chromosome 13q14, was disrupted by specific translocations or deletions in CLL patients resulting in decreased expression of the two mature miRNAs encoded by this locus (Calin et al., 2002). Soon later, mapping of human miRNAs showed that many of them locate in fragile regions in the genome suggesting that miRNA alterations could be a more general defect in tumor cells (Calin et al., 2004). Genome-wide transcriptional analysis later

showed that the profile of miRNA expression was sufficient to classify a variety of human tumors suggesting the putative diagnostic value of these non-coding RNAs (Lu et al., 2005). miRNA profiles can in fact discriminate different subtypes of a particular cancer or even specific oncogenic abnormalities and they are considered as useful biomarkers in multiple diseases (Iorio and Croce, 2012).

The relevance of miRNAs in tumor development has been analyzed in detail in the last decade. Several miRNAs are now to act as oncogenes or tumor suppressor genes by modulating the levels of specific proteins with tumor suppressor or oncogenic potential, respectively (Table 1). In addition, miRNAs are involved in virtually all cellular processes that modulate malignant transformation of cells including the control of the cell cycle (Bueno and Malumbres, 2011), response to DNA damage (Wan et al., 2011), differentiation and stemness (Aberdam et al., 2008), epithelial-to-mesenchymal transition (Brabletz and Brabletz, 2010) or metastasis (White et al., 2011). Recent evidences indicate that miRNAs not only act as internal messengers, but they can also be secreted and circulate in the blood as part of apoptotic bodies, microvesicles, and exosomes or bound to the RNA-binding proteins (Kosaka and Ochiya, 2011). At least some of these extracellular miRNAs may act as a death signal in a competitive process against cancer cells (Kosaka et al., 2012).

The number of miRNAs involved in human cancer is growing rapidly. To illustrate the mechanism of action of these small sequences, a few representative examples of miRNAs with critical roles in tumor development or metastasis are discussed in the next paragraphs.

2.1 miR-15/16 family

As described above, miR-15a and miR-16-1 were the first miRNAs for which a cancer-associated mutation was found (Calin et al., 2002). Deletion of the miR-15a-16-1 cluster in mice causes development of B cell lymphoproliferative disorders, recapitulating the spectrum of CLL-associated phenotypes observed in humans (Klein et al., 2010). These two miRNAs belong to the same family and promote the normal apoptosis response by targeting the anti-apoptotic gene Bcl2 and activating the intrinsic apoptosis pathway (Cimmino et al., 2005). Further studies provided increasing evidence that the miR-15 family regulates the cell cycle through different targets (Linsley et al., 2007). These include multiple interphase cyclins such as cyclin D1, cyclin D2 and cyclin E1 and the expression of these miRNAs induced cell cycle arrest in an retinoblastoma (pRb)-dependent manner (Bandi et al., 2009; Bueno and Malumbres, 2011). miR-15 family members also regulate cellular metabolism and stress, as well as angiogenesis (Finnerty et al., 2010).

2.2 let-7 family

The let-7 family (let-7a-1, a-2, a-3, b, c, d, e, f-1, f-2, g, i and miR-98) is organized in eight distinct clusters located at fragile sites associated with human cancers (Calin et al., 2004). Many of these miRNAs are downregulated in human malignancies and they may function as strong tumor suppressors by targeting major oncogenes such as Ras or Myc (Bueno et al., 2011; Johnson et al., 2005; Roush and Slack, 2008). let-7 also targets a few cell cycle regulators such as Cdc25A, cyclin D2 and Cdk6 thus inhibiting G1/S progression and cell proliferation (Johnson et al., 2007). High mobility group A2 (Hmga2), which is frequently

altered in several cancers, is also another target of let-7 miRNAs. Tumor-associated disruption of let-7 target sites in the HmgA2 3'-UTR impairs this regulation and enhances oncogenic transformation (Lee and Dutta, 2007; Mayr et al., 2007). let-7 miRNAs display multiple other roles in the control of several cellular processes including survival by modulating the levels of Nf2 or caspases (Babashah and Soleimani, 2011; Roush and Slack, 2008). Not surprisingly, let-7 (decreased expression) and miR-21 (increased expression; see below) are among the miRs most frequently associated with poor outcome in human cancer (Nair et al., 2012).

2.3 miR-155

miR-155 was the first miRNA proposed as an oncogene. miR-155 is encoded by the *BIC* (B-cell integration cluster) transcript, a sequence highly expressed in pediatric Burkitt's lymphoma, Hodgkin's disease, CLL, acute myelogenous leukemia (AML), pancreatic tumors, and lung and breast cancer. miR-155 transgenic mice develop acute lymphoblastic leukemia/high-grade lymphoma (Costinean et al., 2006). This miRNA has an essential function in regulating T helper cell differentiation and the germinal center reaction to produce an optimal T cell-dependent antibody response, at least in part, by regulating cytokine production (Thai et al., 2007). miR-155 downregulates Ship and c/EBPbeta, among other targets (Table 1), leading to the expansion of pre-B cells. This miRNA also participates in other cellular processes such as interferon production in natural killer cells, lymphoma cell motility, dendritic cell differentiation and function, skeletal muscle differentiation, or the control of the Tgf β , Jak-Stat, and FoxO3a signaling pathways to modulate migration and invasion in breast cancer cells (Dagan et al., 2012; Leng et al., 2011; Tili et al., 2009; Trotta et al., 2012; Turner et al., 2011; Wang and Hua, 2012).

2.4 miR-17-92

The cluster miR-17-92 (containing seven homologous miRNAs: miR-17-3p, miR-17-5p, miR-18a, miR-20a, miR-19a, miR-19b-1 and miR-92a1) is frequently amplified and overexpressed in hematopoietic malignancies (Ota et al., 2004). This cluster was proposed to function as a strong oncogene by cooperating with Myc and preventing apoptosis (He et al., 2005). Myc actually induces the expression of this oncogenic miRNA cluster, and the mature miRNAs expressed repress E2F transcription factors in a complex network and controls cell cycle progression and apoptosis (O'Donnell et al., 2005). miRNAs in the miR-17-92 cluster can modulate the expression of a wide variety of molecules involved in several cellular processes including apoptosis (Bim), tumor suppression (Pten, Rb2), cell cycle (p21^{Cip1}), cytokine signaling, etc (Babashah and Soleimani, 2011; Mendell, 2008; Olive et al., 2010).

2.5 miR-21

miR-21 is a unique molecule in that it is overexpressed in most tumor types including lung, breast, pancreatic, esophageal, cervical, brain and colon cancers (Table 1). In glioblastoma cells, miR-21 functions as an anti-apoptotic factor and this role is at least partially due to the repression of a highly interconnected tumor suppressor network that includes multiple components of the p53, TGF β and apoptotic pathways (Chan et al., 2005; Papagiannakopoulos et al., 2008). Inhibition of miR-21 results in upregulation of the cell

cycle inhibitor p21^{Cip1} and growth inhibition. Several other tumor suppressors such as Pten or Pdcd4 are also regulated by miR-21 in different cancer cell types (Asangani et al., 2008; Frankel et al., 2008; Meng et al., 2007). Meng et al. showed that inhibition of miR-21 in liver cancer cells decreased tumor cell proliferation as well as migration and invasion. The effects of miR-21 in migration and invasion have been validated in multiple systems and this miRNA is thought to be a major player in metastasis (White et al., 2011). miR-21 can target the inhibitors of matrix metalloproteases Reck and Timp3, resulting in increased activity of these proteolytic enzymes involved in metastasis. Overexpression of miR-21 in mice leads to a pre-B malignant lymphoid-like phenotype (Medina et al., 2010). When miR-21 was inactivated, the tumors regressed completely in a few days, partly as a result of apoptosis. Thus, tumors can become addicted to miRNAs and these data support efforts to use miRNAs as targets in human cancer (see below).

3 Epigenetic alteration of miRNAs in cancer

Epigenetic regulation generally falls into two categories: DNA methylation and histone modifications. Both types of regulation cooperate tightly and histone deacetylases (HDACs) frequently associate with DNA methyltransferases and with methyl CpG-binding domain proteins (MBDs). In many cases, both modifications work together and effective transcriptional reactivation of certain genes cannot be achieved unless both DNA methylation and HDAC-dependent inhibition are eliminated. Epigenetic modifications in the promoter regions of genes and miRNA-dependent regulation at the 3'-UTRs can be considered as two major regulatory mechanisms in eukaryotes. Both DNA methylation and miRNA regulation can suppress gene expression and miRNAs tend to target genes with a low DNA methylation level in their promoter regions, suggesting the complementation between these two regulatory systems (Su et al., 2011).

Recent evidences show that epigenetic mechanisms, including DNA methylation and histone modification, not only regulate the expression of protein-encoding genes, but also affect miRNAs. Indeed, miRNAs undergo the same epigenetic regulatory laws like any other protein-coding gene. The expression of an increasing number of miRNAs is affected by epigenetic aberrations in tumor cells leading to the overexpression or downregulation of these molecules in specific tumor types (Table 2).

3.1 DNA methylation of miRNAs

Approximately half of miRNA genes are associated with CpG islands and their expression can be therefore modulated by methylation (Esteller, 2007; Weber et al., 2007). Accumulated evidences suggest that more than 100 miRNAs are indeed regulated by epigenetic mechanisms, and about half of them are known to be methylated in a cancer-specific manner in more than 20 different tumor types (Kunej et al., 2011). miR-127 was the first miRNA found to be epigenetically regulated. Saito et al. observed a strong upregulation of miR-127 after treatment with 5-Aza-2'-deoxycytidine (5-aza) in several cancer cells (Saito et al., 2006). Subsequent pioneer studies showed hypermethylation and down-regulation of miR-124a, and miR-34b-miR-34c in colorectal cancer cells (Lujambio et al., 2007; Toyota et al., 2008), miR-9-1 in breast cancer (Lehmann et al., 2008), and miR-203 in

lymphomas and Philadelphia-positive leukemias (Bueno et al., 2008). Some of these miRNAs are specifically hypermethylated in metastatic cancer suggesting that these epigenetic modifications not only favor tumor development but may also promote progression to aggressive and malignant tumors (Lujambio et al., 2008; Wee et al., 2012; Zhang et al., 2011b).

3.2 Control of miRNA expression by histone modifiers

miRNAs can also be deregulated as a consequence of aberrant expression of specific epigenetic regulators, such as HDACs, or polycomb repressor complexes (PRC1 or PRC2). PRC1 is formed of Cbx, Mph, Ring, Bmi-1 and Me118, whereas PRC2 consists of Ezh2, Suz12 and Eed (Richly et al., 2011). Inhibition of HDACs results in transcriptional changes in ~40% miRNAs expressed in a particular cell line (Scott et al., 2006) although it is difficult to establish a direct role for the epigenetic machinery on the miRNA regulatory sequences. Recent massive studies using ChIP-seq for specific histone marks [(trimethylated histone H3 lysine 4 (H3K4me3), trimethylated histone H3 lysine 27 (H3K27me3), and dimethylated histone H3 lysine 79 (H3K79me2)] in colorectal cancer cell lines found 47 (out of 174) miRNAs as potential targets of epigenetic silencing (Suzuki et al., 2011). 22 of these miRNAs were associated with CpG islands whereas the others were subjected to regulation by epigenetic marks without evidences of deregulated methylation. For those miRNAs methylated in colorectal tumors, DNA demethylation led to increased H3K4me3 and H3K27me3 marks (Suzuki et al., 2011), suggesting the strong interdependence between these two epigenetic mechanisms.

Tumor cells frequently display not only deregulation in DNA methylation patterns but also altered levels of histone modifiers. HDACs are overexpressed in some particular cancers such as CLL and may mediate the epigenetic silencing of several miRNAs such as miR-15a, miR-16, and miR-29b (Sampath et al., 2012). Inhibition of HDACs induce the re-expression of these miRNAs and the subsequent decline in some of their anti-apoptotic targets such as Mcl-1, mitochondrial dysfunction, and induction of cell death (Sampath et al., 2012). In leukemia cells, overexpression of Polycomb proteins results in the epigenetic repression of miR-31, leading to activation of NF- κ B and apoptosis resistance (Yamagishi et al., 2012). Ezh2, a component of Polycomb repressor complex 2 (PRC2), is able to suppress the expression of additional well-characterized tumor-suppressor miRNAs, such as miR-139-5p, miR-125b, miR-101, let-7c, and miR-200b, in liver tumor cells. These Ezh2-silenced miRNAs display a common effect in modulating cell motility and metastasis, suggesting that Ezh2 can epigenetically silence multiple miRNAs to negatively regulate metastasis from liver tumors (Au et al., 2012). The histone demethylase Jumonji/ARID1 B (Jard1B) also contributes to tumor cell proliferation through the epigenetic repression of let-7 tumor suppressor miRNAs. Binding of Jard1B to the let-7e promoter results in the removal of H3K4me3 histone marks associated with active gene expression. Inhibition of Jard1B results in let-7e re-expression and cell cycle arrest (Mitra et al., 2011).

Tumor-associated epigenetic alterations not only result in the downregulation of tumor-suppressor miRNAs but can also induce the expression of specific transcripts. miR-224, for instance, is reciprocally regulated by HDAC1, HDAC3, and histone acetylase protein, E1A

binding protein p300 (EP300). Liver tumors that significantly overexpress EP300 also overexpress miR-224, and the levels of this miRNA can be attenuated through the inhibition of EP300 (Wang et al., 2012).

In other cases, the epigenetic regulators are not altered by themselves in tumor cells but their activity is modified by oncogenes or tumor suppressors leading to the epigenetic deregulation of miRNAs. BRCA1, a well-known tumor suppressor in the DNA repair pathway, can mediate the epigenetic repression of miRNAs to modulating the activity of HDACs. BRCA1 associates with HDAC2, which acetylates histones H2A and H3 in the miR-155 promoter, leading to the repression of this miRNA (Chang et al., 2011).

4 Epigenetic functions of miRNAs

miRNAs are not only modulated by epigenetic regulation as described in the previous section. It is now clear that miRNAs also have specific epigenetic functions. First, a subset of miRNAs controls the expression of important epigenetic regulators, including DNA methyltransferases, HDACs and polycomb group genes. The expression of these miRNAs, known as epi-miRNAs, has therefore important consequences in the epigenetic regulation of multiple cellular pathways and processes. Second, recent evidence suggests that miRNAs may also have direct epigenetic functions by recruiting specific protein complexes to genomic DNA, and specifically to some promoters modulating the expression of the corresponding genes. Thus, miRNAs and epigenetic pathways appear to form a complex regulatory circuit that modulates the expression of an increasing number of genes in the genome. The disruption of this circuit contributes to various diseases including cancer.

4.1 Epi-miRNAs

miRNAs can contribute to the epigenetic landscape of cells by targeting specific epigenetic regulators such as DNA methyl transferases, HDACs or components of the polycomb repressor complexes (PCR1 and PCR2) (Table 3). The first evidence of the existence of epi-miRNAs was obtained in lung cancer cell lines in which the miR-29 family can directly target DNA methyltransferases such as Dnmt3A and -3B (Fabbri et al., 2007). miR-29 reexpression in cancer cells results in inhibition of DNA methyl transferase activity, re-activation of silenced tumor suppressors and inhibition of tumor growth (Fabbri et al., 2007; Garzon et al., 2009). Other miRNAs that directly control the activity of DNA methyl transferases include miR-152 or miR-301 (Table 3). miR-148a, which is regulated by methylation (Table 2) is also able to modulate the levels of DNA methyltransferases (Braconi et al., 2010; Duursma et al., 2008) suggesting the presence of regulatory loops that tightly link miRNAs and DNA methylation.

Several other miRNAs directly target histone modifiers. The catalytic subunit of the PRC2, Ezh2, which mediates epigenetic gene silencing by trimethylating histone H3 lysine 27 (H3K27me3), is targeted by miR-26a, miR-101 and miR-214 (Table 3). miR-203 controls the levels of Bmi-1, a member of the PRC1 (Wellner et al., 2009). HDAC protein levels are also modulated by several miRNAs including miR-1, miR-140 and miR-449a. Again, miR-449a and b are also epigenetically repressed resulting in a negative feedback loop that affects pRb-E2F activity and cell proliferation (Yang et al., 2009).

These regulatory loops are likely to be more common than previously thought and they are likely to control many different pathways. A recent study has shown the relevance of the regulatory connections between miRNAs and Polycomb proteins in cancer (Cao et al., 2011). miR-101 and miR-26a can control the levels of PRC2 (through repression of Ezh2), and PRC2 acts as a repressor of several miRNAs, including miR-181a, miR-181b, miR200b, miR-200c and miR-203. These miRNAs can actually target components of the PRC1, such as Bmi1, linking the activity of both Polycomb complexes through the tri-methylation of H3K27 (PRC2) or the ubiquitination of histone H2 (PRC1), and this pathway leads to the transcriptional repression of multiple target genes (Cao et al., 2011). miR-200b, which is regulated in a PRC2-dependent manner, is also able to target the PRC2 component Suz12 (Iliopoulos et al., 2010), indicating the existence of further regulatory loops for the epigenetic modulation of gene transcription.

A different circuit involves miR199a, the Brm subunit of the SWI/SNF chromatin remodeling complex and the transcription factor Egr1 (Sakurai et al., 2011). The two mature forms of miR199a, miR-199a-5p and miR-199a-3p, are able to target specific sites in the 3'-UTR of Brm. miR-199a miRNAs are overexpressed in prostate or esophageal cancer cells and this correlates with decreased levels of Brm. The transcription factor Egr1, also upregulated in these cells, is able to transcriptionally induce miR-199a miRNAs, suggesting a pathway in which Egr1 induces miR-199a transcripts and miR-199a-5p and miR-199a-3p repress Brm. Interestingly, Egr1 can be repressed in a Brm-dependent manner, closing the regulatory circle. Egr1, miR-199a-5p, and miR-199a-3p are expressed at high levels in Brm-deficient tumor cell lines but only marginally in Brm-expressing tumor cells. These data suggest the presence of a double-negative feedback loop that may offer an explanation for the differential up- or down-regulation of miR-199a-5p and -3p in a variety of tumors (Sakurai et al., 2011).

4.2 Direct epigenetic modulation by miRNAs

Some recent evidences suggest that, besides the regulation of translation of mRNA targets in the cytoplasm, endogenous miRNAs can directly affect gene expression in the nucleus at the transcriptional level, functioning in a critical interface between chromatin remodeling complexes and the genome. Exogenous small interfering RNAs with complementarity to promoter regions have been previously used to induce heterochromatin formation and transcriptional gene silencing (TGS)(Kim and Rossi, 2007). TGS is mediated by Ago proteins, which are well known for their role in miRNA regulation, and mammalian gene promoters display a specific enrichment for miRNA target sites suggesting the possibility that miRNAs could directly modulate promoter activity (Kim et al., 2008; Younger et al., 2009).

Pioneer studies in 2008 provided the first experimental evidences that miRNAs can directly modulate gene expression by targeting the promoter region of specific genes (Figure 2 and Table 4). miR-373 recognizes a specific sequence at the E-cadherin and Cdc2 promoters and induces the binding and transcriptional function of RNA polymerase at these genes (Place et al., 2008). miR-320, on the other hand, is encoded within the promoter region of the Polr3d gene in the antisense orientation, and the mature miRNA can direct the

recruitment of Ago1 proteins and the PRC2 component Ezh2 at the Polr3d promoter, resulting in H3K27 tri-methylation and transcriptional silencing (Kim et al., 2008). miR-423-5p can recognize target sites at the Igsf1 promoter and participates in the recruitment of Ago2 to a non-coding transcript that overlaps the promoter region (Younger and Corey, 2011). These data suggest recognition of nascent ncRNA transcripts as the mechanism that miRNAs use for TGS. More recently, miR-223 has been found to interact with complementary binding sites in the Nfia promoter (Zardo et al., 2012). During granulopoiesis, miR-223 participates in the recruitment of Ago1, Dicer and Polycomb proteins to the Nfi1 promoter, resulting in increased H3K27 tri-methylation and decreased binding of RNA polymerase II or marks associated to active transcription such as H3K4m3 (Zardo et al., 2012).

Finally, an additional example corroborates that miRNAs can also induce promoter activation and not only repression. The cyclin B1 (Ccnb1) promoter contains highly-complementary sites for 21 miRNAs. Among them, miR-744, miR-1186 and miR-466d-3p can actually induce Ccnb1 expression and, conversely, knockdown of endogenous miR-744 leads to decreased Ccnb1 levels (Huang et al., 2012). miR-744 favors the recruitment of Ago1 and RNA pol II to the Ccnb1 promoter, and results in H3K4 tri-methylation at the Ccnb1 transcription start site. Thus, endogenous miRNAs can either induce or repress the expression of specific genes by recognizing specific target sites at the promoter regions. Despite these few examples, it is not yet clear how widespread is this mechanism during different cellular processes or whether to what extent it is deregulated in human disease.

5 Conclusions

An increasing number of miRNAs are regulated epigenetically and, in parallel, multiple miRNAs are able to modulate the protein levels of epigenetic regulators such as DNA methyltransferases, HDACs or components of the polycomb repressor complexes. In addition, the possibility that miRNAs can directly determine epigenetic modifications in promoter regions suggests that the mechanisms of epigenetic and miRNA regulation are not entirely separable, and they cooperate to establish the gene expression profile in particular cells. Either or both of these regulatory mechanisms can be deregulated in human cancer leading to abnormal gene signatures.

We are still far from understanding the global effect that miRNA-epigenetic networks have in a particular cell, during development or in adult homeostasis. Despite the numerous examples for a few miRNAs in cultured cells, we do not have experimental data for most miRNAs and we lack functional studies for each miRNA and its targets in different cell types. Yet, it is surprising how rapidly the miRNA field has provided with useful biomarkers in cancer research and the variety of therapeutic strategies that exploit the manipulation of miRNAs to control tumor cells (Garzon et al., 2010). Human tumors display a general downregulation of miRNAs but are able to discriminate the cell-of-origin even in poorly differentiated cancers (Lu et al., 2005) (Calin and Croce, 2006; Rosenfeld et al., 2008). The diagnostic value is further reinforced by the analysis of tumor-derived circulating miRNAs in the serum (Kosaka et al., 2010). miRNAs can also be used as an independent prognostic factor in multiple malignancies (Slack and Weidhaas, 2008). The miRNAs most frequently

associated with poor outcome are let-7 (decreased expression in patients with cancer) and miR-21 (increased expression)(Nair et al., 2012). miRNAs also allow to discriminate between metastatic or non-metastatic tumors (Dykxhoorn, 2010) (Shi et al., 2010; White et al., 2011). From the epigenetic point of view, the methylation profile of miRNAs is also useful as a prognostic marker for the metastatic behavior of tumors (Lujambio et al., 2008), and this analysis can now be performed in several types of biological samples such as oral rinses, feces and colorectal mucosa fluids (Lopez-Serra and Esteller, 2012).

Modulation of miRNA expression is also a promising novel strategy in cancer therapy (Garofalo and Croce, 2011; Garzon et al., 2010; Trang et al., 2008). Emergent technologies are aimed to express synthetic miRNAs or to inhibit endogenous miRNAs. The most promising therapeutics include the antagomirs (Krutzfeldt et al., 2005), locked nucleic acid (LNA)-modified oligonucleotides (Orom et al., 2006), anti-miRNA oligonucleotides (AMOs) (Weiler et al., 2006). For miRNAs that function as tumor suppressors, the aim is to restore their expression levels in cancer cells. The expression of miRNAs hypermethylated in tumors can be achieved by using DNA methylation inhibitors such as 5-azacytidine derivatives that are already approved for the treatment of specific hematopoietic malignancies in the USA. However, general epigenetic drugs such as Dnmt or HDAC inhibitors display a dramatic global effect in the genome and they effects are difficult to be attributed to specific coding- or non-coding-gene families. In general, these strategies need to be properly evaluated by in vivo studies using transgenic and knockout mouse strains as has been performed in a few cases (Babar et al., 2012; Kota et al., 2009; Medina et al., 2010; Trang et al., 2010).

Both miRNAs and epigenetics are new chapters in genome biology. We are just starting to understand their in vivo relevance but there is no doubt that their potential in therapeutics opens up new opportunities in the future of cancer therapy.

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Biography

Marcos Malumbres is the head of the Cell Division and Cancer group at the Spanish National Cancer Research Centre (CNIO) in Madrid. His research has been focused to the understanding of the biological relevance of critical cell cycle regulators with potential impact in human disease. Marcos Malumbres has authored more than 100 papers including relevant contributions to understanding the in vivo function of key cell cycle regulators such as Cdks, mitotic kinases and phosphatases or the E3 ubiquitin-ligase APC/C. He has also contributed to deciphering the role of microRNAs in the control of the cell cycle, tumor development and their putative therapeutic uses in cancer.

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Figure 1.

miRNA biogenesis and function. **A**, Similarly to protein-coding genes, miRNAs are expressed by RNA polymerase II in response to different types of transcriptional stimuli and epigenetic modulators. Primary transcripts (prim-miRNAs) are processed by different types of RNases to generate a mature single-stranded miRNA (in red). miRNAs can recognize partially complementary sequences in mRNAs leading to the assembly of RISC complexes containing Ago proteins, and the degradation of these transcripts and/or the repression of their translation. Some of these targets are proteins involved in the epigenetic control of gene expressions generating regulatory circuits with big impact in gene expression. **B**, Recent evidences suggest that miRNAs can also recognize specific target sites in gene promoters, modulating gene expression likely by recruiting similar Ago proteins and chromating remodeling enzymes in the vicinity of promoters.

Figure 2.

Direct effects of miRNAs as epigenetic regulators of gene expression at gene promoters. Three models of action have been reported so far. **A**, Some miRNAs such as miR-373 or miR-744 can function as activators of gene expression by recruiting Ago proteins and non-well established chromatin modifiers to specific complementary sites in gene promoters. **B**, Other miRNAs recruit similar Ago or chromatin modifiers but in this case resulting in the repression of transcription, for instance bringing PRC2 (Ezh2) complexes to the chromatin. **C**, At least in one specific case (miR-423-5p), the miRNA interacts with target sites in an

antisense nascent non-coding RNAs. The recruitment of similar repressor complexes results in repression of neighbor promoters.

Table 1

Representative miRNAs with oncogenic or tumor suppressor activity

miR	Activity	Targets	Deregulation in cancer
let-7 family	Tumor suppressor	Ras, Myc, HmgA2	Downregulated in multiple solid tumors and hematopoietic malignancies
miR-15-16 cluster	Tumor suppressor	Bcl1, Cyclin D1, Wnt3A	Translocated and downregulated in hematopoietic malignancies; downregulated in pituitary, prostate and pancreatic tumors
miR-17-92 cluster	Oncogenic	p63, E2F1, p21 ^{Cip1} , Bim	Upregulated in lung and colon cancer, as well as lymphoma, medulloblastoma and multiple myeloma
miR-21	Oncogenic	Pten, Pdcd4	Overexpressed in multiple solid tumors
miR-34 family	Tumor suppressor	Cyclin E2, Met, Bcl2, MycN, Notch1/2, Cdk4/6	Downregulated in pancreatic cancer and Burkitt's lymphoma.
miR-106b-93-25 cluster	Oncogenic	p21 ^{Cip1} , Bim	Overexpressed in multiple solid tumors and multiple myeloma
miR-155	Oncogenic	Ship, c/EBPbeta, Pu.1, Cutl1, Pcalm	Upregulated in breast, lung, colon and pancreatic tumors and hematopoietic malignancies
miR-203	Tumor suppressor	Abl, p63	Downregulated in multiple solid tumors and hematopoietic malignancies
miR-221 & miR-222	Oncogenic	Pten, Timp3, p27 ^{Kip1} , p57 ^{Kip2} , Bim, Ddit4, FoxO3A	Upregulated in multiple solid tumors and in CLL

Table 2

Epigenetic alteration of microRNAs in human tumors

miRNA	Targets	Cancer	Epigenetic alteration
let7-a-3	Ras, Igf2BP1-3	Colorectal, lung, ovarian	Differential methylation
miR-1	FoxP1, Met, Hdac4	Colorectal, liver	Hypermethylated
miR-9	NFKB1, claudin, E-cadherin	Multiple solid and hematological tumors Breast metastasis	Hypermethylated
miR-34 family	Cdk6, Myc, E2F3, CREB, Notch1, Bcl2	Multiple solid and hematological tumors Metastasis	Hypermethylated
miR-107	Cdk6	Pancreatic	Hypermethylated
miR-124a	Cdk6, cEBPalpha, Vim, SmyD3	Multiple solid and hematological tumors	Hypermethylated
miR-126	N.R.	Bladder, prostatic, lung	Hypermethylated. Repressed by H3K9me2, H3K9me3, and H3K27me3
miR-127	Bcl6	Bladder, prostate	Hypermethylated
miR-129-2	Sox4	Gastric, endometrial, colorectal	Hypermethylated
miR-137	Cdk6, E2F6, NcoA2, Lsd-1	Oral, colorectal, glioblastoma	Hypermethylated
miR-141-200c	Zeb1, Zeb2	Colorectal, breast, lung	Hypermethylated
miR-148a	Tgif2	Multiple solid and hematological tumors. Breast metastasis	Hypermethylated
miR-152	Dnmt1	Endometrial, breast, ALL	Hypermethylated
miR-181 family	Plag1, Notch4, K-Ras	Gastric	Hypermethylated
miR-193a	E2F6, Ptk2, Mcl1	AML	Hypermethylated
miR-199a	Podxl, Ikkbeta	Testicular, ovarian	Hypermethylated
miR-200a/b/429	Zeb1, Zeb2, Sox2, Klf4,	Prostate, liver, ALL	Hypermethylated. Repressed by PRC2 and Hdac4
miR-203	Abl, Bcr-Abl, Bmi1	Multiple solid and hematological tumors	Hypermethylated
miR-223	NFI-A, Mef2C	ALL, AML	Repressed by AML1/ETO
miR-335	Sox4, Tnc	Breast	Hypermethylated
miR-342	PdgfrA, RasA1	Colorectal	Hypermethylated
miR-370	Map3k8	Cholangiocarcinoma	Hypermethylated
miR-449a,b	Hdac1, Cdk6, Cdc25A	Prostate	Repressed by H3K27me3
miR-512-5p	Mcl1	Gastric	Hypermethylated

Data from (Aguirre et al., 2012; Iorio et al., 2010; Kunej et al., 2011; Lopez-Serra and Esteller, 2012; Sato et al., 2011; Valeri et al., 2009). ALL acute lymphoblastic leukemia, AML acute myeloid leukemia.

Table 3

epi-miRNAs regulating effectors of the epigenetic machinery

miRNA	Target	Cancer or tissue	References
miR-1	Hdac4	Skeletal muscle tissue	(Chen et al., 2006)
miR-26a	Ezh2	Lymphoma	(Sander et al., 2008)
miR-29a/b/c	Dnmt3a, Dnmt3b, Dnmt1, YY1	Lung, AML	(Fabbri et al., 2007) (Garzon et al., 2009) (Wang et al., 2008)
miR-101	Ezh2	Prostatic, Bladder	(Varambally et al., 2008) (Friedman et al., 2009)
miR-128	Bmi1	Glioma, pancreatic and colorectal cancer	(Wellner et al., 2009) (Godlewski et al., 2008)
miR-132	MeCP2	Myofibroblast	(Mann et al., 2010)
miR-140	Hdac4	Osteosarcoma and colon cancer	(Song et al., 2009; Tuddenham et al., 2006)
miR-148a/b	Dnmt3b, Dnmt1	Cervical, cholangiocarcinoma	(Duursma et al., 2008) (Braconi et al., 2010)
miR-152	Dnmt1	Endometrial, liver, cholangiocarcinoma	(Tsuruta et al., 2011) (Huang et al., 2010)
miR-185	Dnmt1	Glioma	(Zhang et al., 2011a)
miR-199a	Bmi1	Cancer cell lines	(Sakurai et al., 2011)
miR-200a,b	Suz12, Sirt1, Hdac4	Liver tumors	(Iliopoulos et al., 2010) (Eades et al., 2011) (Yuan et al., 2011)
miR-203	Bmi1	Pancreatic and colorectal cancer cells	(Wellner et al., 2009)
miR-214	Ezh2	Skeletal muscle and embryonic stem cells	(Juan et al., 2009)
miR-301	Dnmt1	Cholangiocarcinoma	(Braconi et al., 2010)
miR-449a	Hdac1	Prostatic	(Noonan et al., 2009)

AML acute myeloid leukemia

Table 4

miRNAs regulating gene expression at the promoter regions

miRNA	Cell type or cellular process	Type of regulation and Target	References
miR-223	Granulopoiesis	Repression of Nfia	(Zardo et al., 2012)
miR-320	Several cell lines	Repression of Polr3d	(Kim et al., 2008)
miR-373	PC3 prostate cancer cell line	Induction of E-cadherin, Csd2	(Place et al., 2008)
miR-324-5p	Breast cancer cells	Repression of Progesterone receptor, Igsf1	(Younger and Corey, 2011)
miR-744 (miR-1186, miR466d-3p)	NIH 3T3 murine fibroblasts	Induction of Cyclin B1	(Huang et al., 2012)