Dysregulation of microRNAs in cancer: Playing with fire

Sonia A. Melo, M. Esteller

MicroRNAs [1] have emerged as key post-transcriptional regulators of gene expression, involved in various physiological and pathological processes. It was found that several miRNAs are directly involved in human cancers, including lung, breast, brain, liver, colon cancer and leukemia. In addition, some miRNAs may function as oncogenes or tumor suppressors in tumor development. Furthermore, a widespread down-regulation of miRNAs is commonly observed in human cancers and promotes cellular transformation and tumorigenesis [2–5]. More than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites, frequently amplified or deleted in human cancer, suggesting an important role in malignant transformation. A better understanding of the miRNA regulation and misexpression in cancer may ultimately yield further insight into the molecular mechanisms of tumorigenesis and new therapeutic strategies may arise against cancer.

Here, we discuss the occurrence of the deregulated expression of miRNAs in human cancers and their importance in the tumorigenic process.

1. Introduction

For the past two and a half decades it has been thought that cancer is caused by genetic and/or epigenetic alterations to oncogenes or tumor-suppressor genes. Various regulatory factors control the expression of these genes, allowing for the correct execution of processes such as division, differentiation, and apoptosis. In cancer, however, a deregulation of these genes causes these processes to become uncontrolled, resulting in tumor formation. Recent research has unraveled molecular mechanisms and damaged genes involved in cancer. One such example is the discovery of microRNAs [6], that ended up in a escalation in research on these RNA molecules as key players in cancer biology. Smaller than protein-coding genes, miRNAs can regulate the translation of hundreds of genes through sequence-specific binding to mRNA [7], and depending on the degree of complementarity will result in the inhibition of translation and/or enhanced mRNA decay [7,8]. In mammals, miRNAs are predicted to control the activity of more than 60% of all protein-coding genes [9] and participate in the regulation of almost every cellular process investigated to date (reviewed in References [10–12]).

In 1998, Fire and Mello established dsRNA as the silencing trigger in Caenorhabditis elegans [13]. The first miRNA to be discovered, lin-4, was identified in C. elegans in a screen for genes that are required for post-embryonic development [14]. The lin-4 locus produces a 22 nucleotide RNA that is partially complementary to sequences in the 3′UTR of its regulatory target, the lin-14 mRNA [15]. Structurally miRNAs are small non-coding regulatory RNAs ranging in size from 19 to 24 nucleotides (see miRBase, http://microrna.sanger.ac.uk/), that potentially target up to one-third of human coding genes making their role in cellular biology even more apparent [16]. These small RNAs post-transcriptionally repress gene expression by recognizing complementary target sites most often in the 3′ untranslated region (UTR) of target messenger RNAs (mRNAs) [17–19]. However, animal miRNAs may also target 5′ UTR and coding regions of mRNAs, as documented by experiments involving both artificial and natural miRNAs and also by bioinformatic predictions [20–22]. MicroRNAs silence the expression of the target mRNAs, either by mRNA cleavage or by translation repression. Nevertheless, it has been described that miRNAs can also increase the expression of a target mRNA [23]. Each miRNA may target several different mRNAs and, conversely, a single mRNA can be targeted by several miRNAs. Furthermore, it was shown that miRNAs can target not only messenger RNA but also DNA; MiR-373 was found to target promoter sequences and induce gene expression [24]. More recently it was described that miRNAs can also target proteins. Eiring et al. reported a novel function of miRNAs called “decoy activity”. MiR-328 interacts with a heterogeneous ribonucleoprotein, hnRNP-E2, to regulate RNA-binding protein function [25].
In this review, we briefly describe miRNA biogenesis and regulation of miRNAs at transcriptional and post-transcriptional levels. Then we focus on miRNAs deregulation in cancers by outlining their roles as oncogenes or tumor-suppressors, their control of multiple cancer-related biological pathways and their epigenetic transcriptional control in human cancers. Finally, we finish this review with a discussion of the potential application of miRNAs as biomarkers, diagnosis, and potential therapeutic tools for human cancers.

2. Biogenesis and maturation of microRNAs

Biogenesis of a miRNA begins with the synthesis by Pol II of a long transcript known as pri-miRNA (Fig. 1). Also Pol III was initially believed to mediate the transcription of miRNAs because it produces some of the other shorter non-coding RNAs: tRNAs, 5S ribosomal RNA and U6 snRNA. Several evidences seem to indicate that pri-miRNAs with their own promoters must be Pol II products [26–30]. However, other pathways generate a minor set of miRNAs, especially from genomic repeats. For example, RNA polymerase III is responsible for transcription of miRNAs in Alu repeats [31].

The first step of miRNA maturation is enzymatic cleavage by the RNase III Drosha which releases a small hairpin that is termed a pre-miRNA of ~70 nucleotides. The RNase Drosha works together with its interacting partner DGCR8 (DiGeorge syndrome critical region gene 8) [32–34]. Drosha belongs to a family of double stranded RNA specific ribonucleases. The dsRNA-binding protein DGCR8 recognizes the stem and the flanking single-stranded RNA (ssRNA) and serves as a ruler for Drosha to cut the stem releasing the hairpin pre-miRNA [35]. Interestingly, the DGCR8 gene is one of the few genes located in a region (chromosome 22) deleted in a genetic disease termed DiGeorge syndrome [34]. This pri-miRNA processing complex of Drosha and DGCR8 is called the Microprocessor [33,34]. Pre-miRNAs have a two-nucleotide overhang at their 3' ends and a 5' phosphate group, which are indicative of their production by an RNase III [32,36]. All the components of this microprocessor are needed for pri-miRNA processing in vivo, as a reduction on the level of either Drosha or DGCR8 by RNAi led to the reduction of both pre-miRNAs and mature miRNAs [33,34]. A few pre-miRNAs are produced by the nuclear pre-mRNA splicing pathway instead of through processing by Drosha. These pre-miRNA-like introns, termed mirtons, are spliced out of mRNA precursors. This class of miRNAs bypass Drosha requirement by taking an alternative biogenesis pathway [37–39].

The nuclear export protein Exportin 5 carries the pre-miRNA to the cytoplasm bound to Ran, a GTPase that moves RNA and proteins through the nuclear pore [40,41]. Yi et al. (2003) demonstrated that the nuclear export is dependent on the exportin-5 nuclear export factor which is a member of the karyopherin family of nucleocytoplasmic transport factors. As with other nuclear transport receptors, XPO5 binds cooperatively to its cargo and the GTP-bound form of the cofactor Ran in the nucleus, and releases the cargo following the hydrolysis of GTP in the cytoplasm (Fig. 1) [40,41]. Pre-miRNAs transported to the cytoplasm are subsequently converted to mature duplex miRNA by another RNase III enzyme, DICER1 [42]. DICER1 is a highly conserved protein with one homologue in the yeast Schizosaccharomyces pombe (Dcr), one in human, one in nematode worm (DCR-1), two in Drosophila (DCR-1 and DCR-2), and four in Arabidopsis (DCL1, DCL2, DCL3, DCL4; [43,44]. Dicer cleavage generates a duplex containing two strands, termed miRNA and miRNA*, corresponding to the two sides of the base of the stem [45,46]. DICER1 knock-out (Dcr−/−)

![Fig. 1. Depiction of the microRNA processing machinery.](image-url)
mice and cells are not viable, indicating a major role for this protein during development and normal cell function [47]. In human cells, the drsBP that associates with Dicer1 is the Trans-activator RNA (tar)-binding protein, TRBP. This protein is required for RNAi function mediated by both siRNAs and miRNAs [48–50], where it acts as a biosensor selecting the drsRNA to be loaded into the RISC [51,52].

Following Dicer1/TRBP cleavage, the resulting ~22-nt RNA duplex is loaded onto an Ago protein so as to generate the effector complex, RISC [48]. One strand of the ~22-nt duplex remains in Ago as a mature miRNA (the guide strand or miRNA), whereas the other strand (the passenger strand or miRNA*) is degraded. Nucleotides 2–7 of the mature miRNA sequence create the “seed region” [53–56] that primarily specifies the target mRNA that the miRNA will bind to.

Although it has been recognized and predicted before that genes can be targeted by multiple miRNAs [57,58], this problem has not been tackled experimentally. A new study by Wu et al. (2010) represents the first defined example wherein multiple miRNAs target the same gene [59]. In this study, Wu et al. experimentally demonstrate through a high-throughput luciferase reporter screen that p21Cip1/Waf1 can be directly targeted by nearly 28 microRNAs (miRNAs).

3. Transcription and post-transcriptional regulation of microRNAs

Transcription is a major point of regulation in miRNA biogenesis. Almost 50% of miRNA genes are located in the introns of protein-coding genes or long non-coding RNAs transcripts, whereas the remainder are independent transcription units with specific promoter elements and polyadenylation signals reviewed in [60]. Approximately 50% of mammalian microRNA loci are in close proximity with other miRNAs. These clustered miRNAs are transcribed as policistronic messages in single transcript units or are overlapped in the host transcripts, within exons or introns, depending on the splicing patterns of the host gene. Numerous Pol II-associated transcription factors are involved in transcriptional control of miRNA genes. For instance, myogenic transcription factors, such as myogenin and myoblast determination 1 (MYOD1), bind upstream of mir-1 and mir-133 loci and induce the transcription of these miRNAs during myogenesis [61–63].

Another clear example is the activation of miRNAs transcription by the tumor suppressor p53. P53 activates the mir-34 family of miRNAs, whereas the oncogenic protein MYC transactivates or represses a number of miRNAs that are involved in the cell cycle and apoptosis [4,64]. Epigenetic control also contributes to miRNA gene regulation [65–67].

Drosha processing constitutes another important point of regulation. It was proposed that SMAD proteins activated by BMP/TGFβ interact with Drosha and DDX5 (also known as p68) to stimulate Drosha processing, although the detailed mechanism for this remains unclear [68]. Drosha processing of pri-miR-18a is dependent on the heterogeneous ribonucleoprotein particle A1 [69]. The number of these regulatory factors is unknown, but it is plausible that nuclear RNA-binding proteins influence miRNA processing through specific interactions with a subset of pri-miRNAs.

The let-7 miRNAs also show interesting expression patterns [70]. The primary transcript of let-7 (pri-let-7) is expressed in both undifferentiated and differentiated ES cells, whereas mature let-7 is detected only in differentiated cells, indicating that let-7a might be post-transcriptionally controlled [71]. Similar post-transcriptional inhibition of let-7 also takes place in tumor cells [72]. Recent studies have shown that an RNA-binding protein, LIN28, is responsible for the suppression of let-7 processing [73–75]. Several different mechanisms of LIN28 activation have been proposed: blockage of Drosha processing [73], interference with Dicer1 processing [75], and terminal uridylation of pre-let-7. Given the cytoplasmic localization of LIN28, and its strong interaction with pre-let-7 (but not with pri-let-7), LIN28 is likely to function mainly in the cytoplasm by interfering with pre-let-7 processing and/or by inducing terminal uridylation of pre-let-7. The U tail that is added to the 3′ end of pre-let-7 blocks Dicer1 processing and facilitates the decay of pre-let-7.

Turnover of miRNAs is a largely unexplored area. RNA decay enzymes might target not only mature miRNAs but also the precursors (pri-miRNAs and pre-miRNAs). Once bound to Ago proteins, mature miRNAs seem to be more stable than average mRNAs; the half-life of most miRNAs is greater than 14 h [76].

RNA editing is another possible way of regulating miRNA biogenesis [77,78]. The alteration of adenosine to inosines, a reaction that is mediated by adenosine deaminases (ADARs), has been observed in mir-142 and mir-151 [79,80]. Because the modified pri-miRNAs or pre-miRNAs become poor substrates of RNase III proteins, editing of the precursor can interfere with miRNA processing. Editing can also change the target specificity of the miRNA if it occurs in miRNA sequences [80].

An increasing number of miRNAs are controlled at the post-transcriptional level [77]. Mir-136 is specifically expressed in neuronal cells, while its expression is suppressed at the Dicer1 processing step in non-neuronal cells [81]. Human miR-31, mir-128 and mir-105, however, might be controlled at the nuclear export step because the precursors are retained in the nucleus without producing mature miRNA in certain cell types. Mature mir-7, mir-143 and mir-145 show reduced expression in cancer cells compared with normal tissue, although the precursor levels are similar between the tumor and normal tissues, which suggests that post-transcriptional deregulation occurs in cancer cells. Thomson et al. (2006), has described the lack of correlation between pri-miRNA and mature expression in the tumor samples, while the normal tissue samples had positive correlation. This demonstrates that the miRNA alterations that occur in tumors are not due to deregulated transcription but can be, in part, due to post-transcriptional regulation of miRNAs [72]. This data suggest a multistep model for the control of miRNA expression. Transcription of the pri-miRNA can be regulated, as has been demonstrated for tissue-specific miRNAs [61,82–84]. Processing at the Dicer step can be delayed or inhibited [42,81].

All together, these observations indicate that the miRNAs can be regulated at various levels, from stability, processing, sequence identity and binding to target mRNAs. Therefore, these regulatory pathways are susceptible of being altered in cancer cells.

4. MicroRNA deregulation in cancer

Like transcription factors, miRNAs regulate diverse cellular pathways and are widely believed to regulate most biological processes. Recent studies have reported the involvement of both genetic and epigenetic mechanisms in miRNA deregulation that can potentially lead to cancer development [85]. Genetic mechanisms are usually chromosomal abnormalities that can lead to the deletion, amplification, or translocation of miRNAs. In addition, approximately 50% of all annotated human miRNA genes are located at fragile sites or areas of the genome that are associated with cancer which are prone to breakage and rearrangement in cancer cells [86–88]. For example, mir-15a and mir-16-1, two tumor-suppressor microRNAs, are severely down-regulated in 70% of patients with chronic lymphocytic leukemia (CLL) due to chromosomal deletions or mutations at the 13q13.4 loci where they are situated [86].
In addition to genomic and epigenetic alterations [87,89,90], miRNA deregulation in cancer might be attributable to the impairment of microRNA-processing steps [5,72,91,92], like the described mutations in the TARBP2 gene that lead to DICER1 destabilization and therefore to a global down-regulation of miRNAs [5]. In accordance with these results a widespread decrease in mature miRNAs is often observed in various human malignancies [2,89,93,94]. From these observations a new pathway could be emerging for colorectal tumorigenesis, in addition to the classical "mutator or chromosomal instable (CIN)" categories, standing for a subset of microsatellite instable colorectal tumors bearing mutations in the microRNA machinery genes – mutated microRNA machinery phenotype (MMMP). This molecular subgroup of tumors claims to group up colorectal cancers with MSI and is characterized for exhibiting concordant tumor-specific gene mutations in microRNA machinery genes, in this way deregulating the cells miRNAome. Even though larger prospective studies will be required to fully characterize and validate this feature as a classificatory criterion, the conductive molecular events have been functionally characterized, and it is likely that patients suffering from this mutated microRNA machinery phenotype (MMMP) subset of colorectal tumors would benefit from a broader miRNAome modifying approach.

Transcription factors may induce miRNAs by activating the transcription of pri-miRNAs. Given the wide impact of transcription factors on fundamental cellular processes, it is not surprising that many oncogenes or tumor suppressors function as transcription factors. Many miRNA-transcription factor connections have been discovered in cancer [95]. P53, c-Myc and E2F are further discussed below.

The steady-state level of mature miRNA is determined not only by the transcription rate of the pri-miRNA but also by the processing efficiency of its precursors and by its stability. MiRNAs often exhibit a discrepancy in expression of the mature form, relative to that of the precursor [81,96–100]. Although miRNAs in a genomic cluster are usually expressed from a common pri-miRNA, the levels of individual miRNAs in the cluster are not necessarily the same [101,102]. A time-course experiment, after induction of pri-miR-21, revealed delayed kinetics in accumulation of mature miR-21 [103]. Collectively, these observations indicate that miRNA processing and stability are important factors that determine miRNA expression level.

The expression levels of DICER1 or Drosha are altered in a number of cancers [310–107]. Drosha up-regulation is seen in more than half of cervical squamous cell carcinoma (SCC) specimens, and is likely due to the copy number gain at chromosome 5p, where the Drosha gene is located [106]. Hierarchical clustering of miRNA expression data successfully classified cervical SCC samples into two groups according to Drosha overexpression. Notably, some miRNAs were reduced upon Drosha overexpression, indicating that individual miRNAs respond differently to an overexpression of the miRNA processing machinery. Interestingly, Drosha was reported to interact with an oncogenic fusion protein derived from a chromosomal translocation in some leukemias [108]. This interaction affects pri-miRNA selection of Drosha and, as a result, influences miRNA expression patterns. Moreover, frequent hemizygous deletion of DICER1 occurs with a high incidence rate in breast tumors [109].

Findings from two mouse models strongly suggest that alterations in miRNA expression alone can cause a cell to become neoplastic. The miR-155 developed acute lymphoblastic/high-grade lymphoma [110], while the knock-out model of the tumor-suppressor cluster miR-15/16 developed chronic lymphocytic leukemia [111].

Recognition of miRNAs that are differentially expressed between tumor tissues and normal tissues may help to identify those miRNAs that are involved in human cancers and further establish the key role of miRNAs in the tumorigenic process.

5. Epigenetic control of microRNAs expression

Three main epigenetic events regulate tumor-associated genes: the aberrant hypermethylation of tumor suppressor genes, global DNA hypomethylation and post-translational modifications of histones [67,112–114]. An extensive analysis of genomic sequences of microRNA genes has shown that approximately half of them are associated with CpG islands [115,116]. Therefore these epigenetic events can also affect miRNA expression. In addition, some miRNAs are up-regulated (a) upon the exposure of cells to the agent 5-aza-2'-deoxyctydine [116], (b) upon mutation of methyltransferases (DNMTs) [90], or (c) upon histone deacetylase inhibitor treatment [117]. These studies have identified some miRNAs that are repressed by CpG island hypermethylation in cancers relative to normal tissue. Representative examples include miR-9-1 in breast cancer [116] and miR-124a in colorectal tumors [90]. The miR-203 locus also frequently undergoes DNA methylation in T-cell lymphoma but not in normal T lymphocytes [118]. In the case of miR-124a, hypermethylation is tumor type specific, as no methylation is seen in neuroblastoma. Moreover, epigenetic silencing of a miRNA may be a reflection of tissue specificity. For example, miR-124a is normally highly expressed in neuronal tissues, while its epigenetic repression in colorectal tumors is not surprising [90]. Saio and colleagues have shown that DNA methylation status and chromatin structure around miRNA genes differ between bladder cancer cells and normal human fibroblasts [119]. They further demonstrate that inhibition of DNA methylation and histone deacetylation induce the expression of miR-127 only in cancer cells [119]. The methylation of miR-127 and miR-124a genes influences the expression of two oncogenic proteins (BCL6 and CDK6, respectively), which are not normally regulated by methylation.

Epigenetic silencing of several miRNAs is a frequent and early event in breast cancer [116,120], and although the let-7 family is globally down-regulated in lung cancer [121,122] there is evidence of let-7a-3 hypomethylation [123]; this is perhaps another example of how miRNAs can have bivalent roles in malignancy.

MiRNAs can also counteract CpG methylation. For example, miR-29 directly targets DNMT-3A and -3B [124]. In agreement with this observation, ectopic expression of miR-29 results in a global reduction of DNA methylation, subsequently leading to a depression of tumor-suppressor genes that had been silenced by promoter methylation in cancer cells [124].

In conclusion, epigenetic changes complemented by genetic inactivation due to mutation or deletion are also a possible mechanism that partially account for miRNA deregulation in cancer cells.

6. Consequences of aberrant microRNA expression in cancers

If we compare global gene expression profiles in cancer and normal tissues, we find that many miRNAs and mRNAs are deregulated. Therefore, it is plausible that tumorigenesis and/or malignant progression results from changes in the entire miRNAome, rather than from the change of a single miRNA that regulates an oncogenic (or tumor-suppressive) target gene.

MiRNAs regulate the expression of their targets, so over- or underexpression of miRNAs is expected to result in down- or up regulation, respectively, of the protein product of the target mRNAs. It is not difficult to associate a miRNA with cancer if a direct target of a miRNA is an oncogene or a tumor-suppressor gene. For example, mir-15 and miR-16 are severely down-regulated in 70% of patients with chronic lymphocytic leukemia (CLL) and induce apoptosis by targeting antiapoptotic gene B cell lymphoma 2 (BCL2) miRNA [125], which is a key player in many types of human cancers including leukemias, lymphomas, and carcinomas [126]. Moreover, emerging evidence suggests that miRNA let-7
may play a critical role in lung cancer development. Takamizawa et al. (2004) observed that the expression levels of let-7 were frequently reduced in both in vitro and in vivo lung cancer studies; reduced let-7 expression was significantly associated with shortened postoperative survival, independently of disease state [121]. Johnson et al. (2005) also showed that lung tumor tissues display significantly reduced levels of let-7 and significantly increased levels of RAS protein relative to normal lung tissue, suggesting let-7 regulation of RAS as a mechanism for lung oncogenesis. Since the demonstration that let-7 miRNA directly regulates RAS and MYC oncogenes [127] a number of other miRNA target pairs have been studied.

Breast cancer also presents a deregulated pattern of expression of miRNAs between normal and neoplastic breast tissues. Iorio et al. (2005) found miR-125b, miR-145, miR-21 and miR-155 were significantly reduced in breast cancer tissues. Most importantly, they also observed that expression patterns of miRNAs were correlated with tumor stage, proliferation index, estrogen and progesterone receptor expression and vascular invasion [128]. The differentiation program epithelial to mesenchymal transition (EMT) involves changes in a number of miRNAs. Some of these miRNAs have been shown to control cellular plasticity through the suppression of EMT-inducers or to influence cellular phenotype through the suppression of genes involved in defining the epithelial and mesenchymal cell states. MiR-200 family of miRNAs are profoundly involved in this process and are deregulated in breast cancers [129]. Also miR-10b is highly expressed in metastatic breast cancer cells and positively regulates cell migration and invasion [130].

Colorectal neoplasia is also characterized by alterations in miRNAs expression. MiR-145 and miR-143 are frequently reduced at the adenomatous and cancer stages of colorectal neoplasia [100]. However, it was also described that the levels of pre-miR-143 and pre-miR-145 are not altered in precancerous and neoplastic colorectal tissue, suggesting that post-transcriptional control is the cause for the reduced mature miRNA levels [100].

A reduced expression of miR-26a is observed in hepatocellular carcinoma (HCC) cells, a miRNA that is normally expressed at high levels in diverse tissues. Expression of this miRNA in liver cancer cells in vitro induces cell cycle arrest associated with direct targeting of cyclins D1 and D2. The administration of miR-26a in a mouse model of HCC results in inhibition of cancer cell proliferation, induction of tumor-specific apoptosis, and dramatic protection from disease progression [131]. In a recent cohort study published by Ji et al. (2009) tumors had reduced levels of miR-26 expression, as compared with paired non-cancerous tissues, which indicated that the level of miR-26 expression was also associated with hepatocellular carcinoma. Furthermore patients whose tumors had low miR-26 expression had shorter overall survival but a better response to interferon therapy than did patients whose tumors had high expression of the microRNA [132].

In human testicular germ cell tumors two miRNAs were reported to be oncogenic, miR-372 and miR-373 [133]. miR-372 and miR-373 inhibit p53-mediated CDK inhibition through direct inhibition of the Large Tumor Suppressor Homolog 2 (LATS2), and permitted the proliferation and tumorigenesis of primary human cells which have both oncogenic RAS and active wild-type p53 [133].

As mentioned before, in several types of lymphomas, including Burkitt’s lymphoma, the expression of miR-155 is increased compared to normal cells [134]. Mir-155 is located in the conserved region of the BIC gene and expression of BIC/miR-155 is elevated in Hodgkin and Burkitt lymphoma [134]. Furthermore, miR-155 has been shown experimentally to be a bona fide oncogene, as is ectopic expression accelerates tumor development [64,135].

Some miRNAs appear to be deregulated in cancers much more frequently than others. These miRNAs may play key roles during tumorigenesis. For example, the miR-17-92 cluster is frequently amplified in lymphoma and plays a role as an oncogene, possibly by targeting apoptotic factors activated in response to MYC overexpression [64,83]. The miR-17-92 cluster was also found overexpressed in lung cancer, especially in small-cell lung cancer [136].

7. Aberrant action of microRNAs with no alteration of their expression levels

The function of protein-coding genes is altered by point mutations, which either transform proto-oncogenes to oncogenes or abrogate functions of tumor-suppressor genes. In theory, the same mechanism of activation/inactivation may apply to miRNAs. However, mutation in mature miRNA seed sequence seems to be a rare event [88,137–139].

In contrast, sequence variation in miRNA target sites may occur and play a role in cancer. In silico analyses of expressed sequence tag and single nucleotide polymorphism (SNP) databases indicate different allele frequencies of miRNA-binding sites in cancers versus normal tissues [140]. Several experiments have shown that SNPs in miRNA target sites affect miRNA interaction with its target miRNA and are implicated in disease [138,141–143]. An interesting exemplification of this mechanism is found in let-7 and its target oncogene, HMGA2 [144,145]. Chromosomal rearrangements at the HMGA2 locus in several tumors separate the open reading frame [104] from the 3’UTR that contains let-7 target sites. As a result, HMGA2 escapes from let-7 regulation, is overexpressed, and promotes tumorigenesis [144].

An alternative splicing event may result in a different 3’UTR that displays different miRNA recognition sites, as exemplified in the targeting of Tropomyosin-related kinase C by miR-9, -125a, and -125b. One miRNA isoform encodes a truncated ORF that is dominant negative to the intact protein. In this isoform, the 3’UTR contains the target sites of these miRNAs. In contrast, the target sites are absent in another isoform encoding the intact ORF; only the former isoform was repressed by the miRNAs [146]. Although the stop codon is usually located in the last exon, generation of different 3’UTRs by multiple polyadenylation sites or alternative splicing has been known to occur in a small but significant fraction of genes [147]. Thus, variation of the 3’UTR and of attended miRNA target sites is expected to be a mechanism for oncogene activation or tumor-suppressor inactivation.

Recently, Kedde and colleagues demonstrated that the expression of dead end 1 protein (Dnd1), an evolutionary conserved RNA-binding protein, counteracts the function of several miRNAs in human cells and in primordial germ cells of zebrafish by binding mRNAs and prohibiting miRNAs from associating with their target sites. These effects of Dnd1 are mediated through uridine-rich-rich regions present in the miRNA-targeted mRNAs [148].

Recently, Steitz and colleagues [23] reported that miRNAs activate the translation of the target miRNA in cells arrested at the G0/G1 stage. In addition to the aberrant miRNA expression, the switch from repression to activation should be considered in studying the role of miRNAs in differentiation and tumorigenesis, as the same miRNA may exert opposite effects in quiescent cells and proliferating cancer cells in a given tissue.

8. Oncogenic or tumor-suppressive microRNAs

Having in mind their broad effects, miRNAs have been proposed to function as oncogenes or tumor-suppressor genes given their inhibition of a variety of tumor-suppressive and oncogenic mRNAs, respectively [85,149]. In particular, three distinct mechanisms have been proposed. First, oncogenic miRNAs can undergo a gain
of function in tumors due to genomic amplifications. This has been clearly demonstrated for the miR-1–92 cluster, whose amplification in B-cell lymphomas promotes their development [64,150,151]. Furthermore, tumor-suppressive miRNAs could undergo loss of function in tumors due to chromosomal rearrangements, deletions or mutations. This has been shown for several miRNAs, including the let-7 family, whose expression can impair tumorigenesis through inhibition of oncoproteins like the RAS family and HMG A2 [152,153]. In particular, let-7 family members are in sites of frequent deletion in human tumors, and their processing is inhibited by the oncopgenic Lin28 proteins [73,74,154]. Finally, and on the side of the mRNA targets, oncogenes can acquire mutations to remove miRNA-binding sites in tumors. This has been described for HMG A2, whose translational attenuation promotes lipoma development by releasing the transcript from let-7-mediated tumor-suppression [145].

There are a number of miRNAs that are overexpressed in one type of cancer and down-regulated in another. For example, miR-205 is up-regulated in lung [122], bladder [155], and pancreatic cancers [156]. Contrarily, it is significantly down-regulated in prostate [157], breast cancers [158] and esophageal squamous cell carcinoma [159].

As mentioned before, Eiring et al. has shown that miR-328 can act as a decoy by binding to a regulatory RNA binding protein (hnRNP E2) and preventing it from blocking translation of mRNAs. Thus, miR-328 has a dual role in the regulation of gene expression [25]. These findings are intriguing because a miRNA-mediated regulatory function associated with RNA binding proteins has never been described before.

Therefore, it should it is noteworthy that some miRNAs can have dual oncogenic and tumor-suppressive roles in cancer depending on the cell type and pattern of gene expression [160].

9. Regulation of cell cycle factors by microRNAs in human cancer

Cell cycle regulators frequently act as oncoproteins or tumor suppressors. The cell cycle inhibitor p27(Kip1) is one of the best examples. P27(Kip1) is a tumor suppressor that when mutated predisposes cells to tumorigenesis upon exposure to carcinogens [161], P27(Kip1) binds to Cdk2-cyclin E and prevents G1-to-S transition [162]. P27(Kip1) mRNA transcript is a direct target of miR-221 and -222 in glioblastomas [163,164] and prostate cancer cells [165]. In these types of cancer, p27(Kip1) expression is inversely correlated with that of miR-221 and -222. MiR-221 and -222 are overexpressed in other cancers, suggesting that they play a role in a wide range of tumors [166]. MiRNAs also regulate other cell cycle proteins including Cdk6, Cdc25A, Ccn d2 (cyclin D2) [167], Cdk4 [168], a Rb-family protein [101], and p180 subunit of DNA polymerase α [62]. It has been described perturbation of the cell cycle by overexpression or inhibition of some miRNAs [3,62,163,168–171]. Nonetheless, it has not been reported alterations of miRNAs expression during normal cell cycle.

The retinoblastoma (pRB) pathway s one of the major cell cycle pathways and is altered in almost every human cancer [172]. pRB is a transcriptionally represses cell cycle transcription factors of the E2F family resulting in a proliferative arrest. This is relieved by pRB phosphorylation by the cyclin-dependent kinases (CDKs), complexes formed by a cyclin and a kinase that trigger progression throughout the different phases of the cell cycle. CDKs are, in addition, negatively regulated by cell cycle inhibitors of the INK4 (such as p16INK4a) or Cip/Kip families (such as p21Cip1 or p27Kip1) [172]. pRB itself is abnormally down-regulated by the overexpression of the miR-106a in different human cancers [173]. P130/RBL2, another member of the pRB family, is controlled by the miR-290 cluster, which regulates the expression of DNA methyltransferases in a p130-dependent manner affecting telomere-length homeostasis [174]. P130/RBL2 is also targeted by the oncopgenic miR-17–92 cluster resulting in a clonal expansion required for the proper differentiation of adipocytes [175]. The positive regulators of the cell cycle, cyclins and CDKs, are also targeted by miRNAs. The protein levels of cyclin D1/CCND1 and CDK6 are down-regulated by miR-34a inducing significant G1 cell cycle arrest in the A549 cell line [176]. The miR-34 family of miRNAs are directly induced by p53 and participate in DNA damage response and oncopgenic stress induced by this tumor suppressor [177]. miR-34 is also able to promote cell cycle arrest by decreasing CDK4 and Ccyclin E/CCNE2 protein levels [168]. CDK6 is also targeted by miR-124 or miR-137, two miRNAs silenced by hypermethylatation in tumor cells of different origins [90,178]. Most of these miRNAs function as tumor suppressors in several malignancies and it is conceivable that they exert their function through multiple targets. Thus, the let-7 family may control multiple regulators of cell proliferation such as cyclin A2, cyclin B1, cyclin E2 and CDK8 among other cell cycle targets [167]. Some other oncopgenic miRNAs may exert their function through the inhibition of cell cycle inhibitors such as members of the INK4 or Cip/Kip families. p16INK4a, a CDK4/6 specific inhibitor, is controlled by miR-24, a miRNA that is down-regulated during replicative senescence [179]. p21Cip1, a p33-target of the Cip/Kip family of cell cycle inhibitors, is a direct target of miR-106b, which is overexpressed in multiple tumor types and plays a critical role in cell proliferation by regulating the G1-to-S cell cycle transition [180]. The p27kip1 protein, a second member of the Cip/Kip family with a relevant role as tumor suppressor in human cancer, is mostly controlled at the post-transcriptional level [163], miR-221 and miR-222 can function as oncogenes in human tumors by binding to target sites in the 3′UTR of p27kip1 inhibiting its translation [163]. The physiological up-regulation of miR-221 and miR-222 coordinates competency for initiation of S phase with growth factor signaling pathways that stimulate cell proliferation [181].

Therefore, disruption of miRNAs expression that target cell cycle proteins, could ultimately lead to the progression of the malignant phenotype in human tumors.

10. MicroRNAs, p53 and programmed cell death

P53, a well known transcription factor, is described as the guardian of the genome owing to its critical role in regulation of the cell cycle and apoptosis upon DNA damage. p53 is the most extensively studied tumor suppressor and its importance is underscored by mutation of p53 in almost 50% of human cancers.

MiRNA profiling after p53 induction pointed at miR-34a, -34b and -34c as the most up-regulated miRNAs [168,169,182,183]. These miRNAs are induced after genotoxic stress in a p53–dependent manner in vitro and in vivo [168,183]. Transcription of pri-miRNAs -34a, -34b and -34c from both loci is directly activated by p53. These miRNAs promote cell cycle arrest, apoptosis, and senescence [168,169,182–185]. These effects are explained by the repression of several direct targets of the miR-34s such as Bcl-2 [185], Cdk6, and Hepatocyte growth factor receptor [168]. In addition to the miR-34s, other miRNAs may be important in the p53 pathway. MiR-30c, -103, -26a, -107, and -182 were clearly induced, albeit less robustly, upon DNA damage in a p53-dependent manner [182]. miR-26a expression was also shown to be dependent on p53 [186]. MiR-504 acts as a negative regulator of human p53 through its direct binding to two sites in the p53 3′ untranslated region [187].

The tumor suppressor protein p53 was also described to modulate miRNA processing through association with p68 and Drosha [188]. Under conditions of DNA damage, several miRNAs, including miR-143 and miR-16, are post-transcriptionally up-regulated [188].
Co-immunoprecipitation studies indicate that p53 is present in a complex with both Drosha and p68, and addition of p53 to in vitro processing assays could enhance Drosha processing [188]. Interestingly, several p53 mutants that have been previously linked to oncogenic progression suppressed miRNA expression [188]. These results indicate that the association of p68/Drosha with accessory factors, such as p53, may be particularly important for the rapid induction of miRNAs in response to extracellular stimuli.

Apoptosis or programmed cell death is an active process controlled by a gene expression program that varies depending on the biological context. Because a balance between proliferation and apoptosis is essential for tissue homeostasis and proper differentiation, deregulation of apoptosis may give rise to cancer. MiRNAs participate in tumorigenesis by directly targeting antiapoptotic genes. Representative examples include the repression of antiapoptotic genes Bcl2 and Bcl2 by miR-29b [97] and by miR-34s [185], -15a, and -16 [125], respectively. The loss of these miRNAs due to mutation of p53 or deletion of chromosome 13q14 leads to an increase in the antiapoptotic gene expression and persistence of tumor cells that would have normally undergone apoptosis. It is very likely that miRNAs target other genes in the apoptotic pathway, as transfection or expression of a number of miRNAs is associated with apoptosis [189–191].

11. The role of miRNAs in invasion and metastasis

Features of malignant tumors, distinct from benign tumors, include invasion and metastasis. Malignant tumors are fatal, mostly due to their capacity to invade neighboring tissues and metastasize through the bloodstream to distant organs. About 90% of cancer-related deaths are caused by the development of malignant tumors distant from the primary site lesions as a result of metastasis [192]. Recent studies have suggested an important role for miRNAs in metastasis formation. We can classify these miRNAs in two major categories: metastatic inducers and metastatic suppressors. Metastatic inducers include miR-10b, miR-21, miR-127, miR-199a, miR-210, miR-373 and miR-520c. Mir-10b down-regulated in a number of cancers [128,193], is unexpectedly found to be up-regulated in about 50% of metastatic breast cancers. Ectopic expression of miR-10b promotes invasion, intravasation and metastasis in otherwise non-invasive or non-metastatic breast cancer cell lines [130]. Moreover, miR-10b directly targets HOXD10, whose reduction induces the expression of a well-characterized pro-metastatic gene, RhoC [130]. Further, this miRNAs have the ability to promote migration, invasion, and metastasis of non-invasive breast cancer cells in vitro and in vivo [130].

Several miRNAs seem to be metastatic suppressors: let-7 family, miR-100, miR-126, miR-218, miR-335. Reduced levels of miR-126 and miR-335 were found in breast cancer characterized by poor metastatic-free survival [194], while significantly decreased expression of miR-let-7c, miR-100 and miR-218 are differentially expressed between metastatic prostate cancer from high grade localized prostate cancer [195]. Moreover ectopic expression of miR-125 impairs cell motility and invasion in a breast cancer cell line [196], and reduction of global miRNA expression enhances migration of cells [3].

Interestingly, of the metastatic inducers miRNAs only miR-21 is a miRNA with established oncogenic properties. MiR-21, one of the most frequently up-regulated miRNAs in cancer, promotes cell motility and invasion by directly targeting PTEN (phosphatase and tensin homolog), a tumor suppressor known to inhibit cell invasion by blocking the expression of several matrix metalloproteases [197]. Another pathway was recently reported in colorectal cancers, where miR-21 promotes invasion, intravasation, and metastasis by downregulating Pdcd4 [198]. Alternatively, the majority of metastatic suppressor miRNAs found to date are also considered tumor suppressor miRNAs. These observations suggest that either metastatic inducers miRNAs uniquely regulate key sets of genes involved in invasion and migration, or that these inducers miRNAs may also have other, yet unknown, tumorigenic properties.

12. The role of microRNAs in angiogenesis

Angiogenesis is the process by which new blood vessels form through the growth of existing blood vessels, and involves the proliferation, sprouting, and migration of endothelial cells, followed by pruning and remodeling of the vascular network. Major promoters of angiogenesis include vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which activate several downstream pathways, including the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3 K) pathways, to regulate cell motility, proliferation, and survival [199]. MicroRNAs are emerging as important modulators of angiogenesis. Additionally, dynamic changes in microRNA expression in response to growth factor stimulation [200,201] [202] or hypoxia [203] imply that microRNAs are an integral component of the angiogenic program.

The stimulation of neovascularization by c-Myc involves a down-regulation of antiangiogenic factor Tsp-1. C-Myc represses Tsp-1 and a related protein, Connective tissue growth factor (CTGF) by activating the miR-17-92 cluster [204]. Tsp-1 and CTGF appear to be direct targets of miR-19 and -18, respectively. In fact, ectopic expression of the miR-17-92 cluster is sufficient for promoting angiogenesis [204]. A recent observation indicates that other miRNAs, miR-378 and -27a, may play a role in angiogenesis [171,205]. Viral miRNAs may also play a role in angiogenesis, as Tsp-1 has been shown to be a direct target of KSHV miRNAs [206]. Furthermore, miR-126 promotes angiogenesis by repressing sprd1 and pik3r2, which normally inhibit VEGF signaling [207,208].

Thus, targeting the expression of microRNAs may be a novel therapeutic approach for diseases involving excess or insufficient vasculature.

13. MicroRNAs as diagnostic tools

Many miRNAs are uniquely and differentially expressed in certain tissues as compared with normal adjacent tissues. These small RNA molecules can have diagnostic or prognostic value, as miRNA expression profiles reflect tumor origin, stage, and other pathological factors. For example, the expression of miRNA let-7 is down-regulated in lung cancer but not in other cancers, such as breast or colon cancer [121,127,209]. MiRNA expression profiles indicate that miRNAs are a better indicator for distinguishing cancer tissues from normal tissues, and can successfully classify even poorly differentiated tumors [2]. These observations suggest that miRNAs can be used as biomarkers and diagnostic tools for cancer detection. Moreover miRNAs can function as accurate molecular markers also because they are relatively stable and resistant to RNase degradation—probably due to their small size [210–212]. They are highly stable in tissue sections and in blood. Thus their relatively easy and reproducible detection makes them good candidates for biomarkers of cancer.

MiRNAs can be isolated and quantified from formalin-fixed paraffin-embedded (FFPE) specimens. qRT-PCR and microarray data were reliable and reproducibly obtained from FFPE samples that had been routinely processed and stored frozen for 10 years. The data from FFPE samples are consistent with those from frozen samples [213,214]. The development of qRT-PCR methods has improved the sensitivity of miRNA detection down to a few nanograms of total RNA. This amount can easily be obtained by fine-needle aspiration
biopsies (FNABs); in fact there has been a report of successful miRNA measurement by qRT-PCR on FNAB samples\[215]. MiRNA markers that could be used for cancer diagnosis are becoming available. For example, miR-196a is high in pancreatic ductal adenocarcinoma (PDAC) but low in normal tissues and chronic pancreatitis [215], miR-217 exhibits the opposite expression pattern [215]. Thus, the ratio between miR-196a to miR-217, calculated by qRT-PCR measurement of the two miRNAs from a tiny amount of total RNA, indicates whether the samples contains PDAC [216]. Once reliable indicator miRNAs are chosen, they will likely yield easy and accurate tools for cancer diagnosis.

14. MicroRNAs as cancer therapeutic tools

For the past two and half decades it has been though that cancer is caused by genetic and/or epigenetic alterations in protein-coding oncogenes and tumor suppressor genes. These findings have informed the development of novel (targeted) therapies that are based on specific genetic alterations involved in cancer pathogenesis. Nonetheless, with the advent of miRNAs era it was discovered that a number of miRNAs affect the growth of cancer cells in vitro and in vivo when overexpressed or inhibited. Because miRNAs function as oncogenes or tumor suppressors, it might be possible to regulate miRNA expression and/or use artificial miRNAs to regulate cancer formation.

Overexpression or inhibition of miRNAs can be achieved in several ways. Synthetic microRNA mimics include siRNA-like oligoribonucleotide duplex [217] and chemically modified oligoribonucleotide [218]. Conversely, miRNAs can be inhibited by various modified antisense oligonucleotides such as 2'-O-methyl antisense oligonucleotide duplex [217] and chemically modified oligoribonucleotide [218].

Table 1

| Human miRNA | Deregulation in cancer |
|-------------|------------------------|
| let-7 family (various) | Down-regulated in lung, breast, gastric, ovary, prostate and colon cancer |
| miR-10a (2q31.1, intergenic) | Down-regulated in AML |
| miR-15a, miR-16-1 cluster (13q14.3, intron 4 non-coding RNA DLEU2) | Down-regulated in CLL, DLBCL, multiple myeloma, pituitary adenoma, prostate and pancreatic cancer |
| miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-17-92 cluster (13q31.3, intron 3 C13orf25) | Up-regulated in nasopharyngeal carcinoma |
| miR-26a (3p22.2) | LOH at miR-17-92 locus in melanoma, ovarian and breast cancer |
| miR-100b-93-25 cluster (7q22.1) | Overexpression in lung and colon cancer, lymphoma, multiple myeloma, medulloblastoma |
| miR-21 (17q23.1, 3’UTR TMEM49) | Down-regulation in hepatocellular carcinomas |
| miR-29 family (various) | Over-regulation in breast cancer |
| miR-34 family (1p36.23, 1q23.1, intergenic) | Overexpression in gastric, colon and prostate cancer, neuroblastoma and multiple myeloma |
| miR-101 (1p31.3, 5p24.1) | Overexpression in glioblastoma, breast, lung, prostate, colon, stomach, esophageal, and cervical cancer, uterine leiomyosarcoma, DLBCL, head and neck cancer |
| miR-122a (1q42.31, intergenic) | Down-regulated in CLL, colon, breast, and lung cancer and cholangiocarcinomas |
| miR-124a family (various) | Up-regulations in breast cancer |
| miR-125a, miR-125b (various) | Down-regulated in pancreatic cancer and Burkitt’s lymphoma |
| miR-127 (14q32, RTL1 exon) | Hypermethylation of miR-14b, c in colon cancer |
| miR-141, miR-145 cluster (intergenic 5q32) | Down-regulation in prostate cancer, hepatocellular carcinoma, and bladder cancer |
| miR-153 (21q22.1, exon 3 miRNA BIC) | Down-regulation in hepatocellular carcinoma |
| miR-181 family (various) | Hypermethylation in colon, breast, gastric and lung cancer, leukemia and lymphoma |
| miR-221, miR-222 cluster (Xp11.3, intergenic) | Down-regulation in glioblastoma, breast, prostate and ovarian cancer |
| miR-200 family (various) | Up-regulation in myelodysplastic syndrome and AML |
| miR-205 family (various) | Hypermethylation in tumor cell lines |
| miR-372, miR-373 cluster (19q13.41, intergenic) | Down-regulated in colon adenoma/carcinoma, in breast, lung, and cervical cancer, in B-cell malignancies |
| miR-203 (1q22.2) | Overexpressed in pediatric Burkitt’s lymphoma, Hodgkin’s lymphoma, primary mediastinal lymphoma, DLBCL, breast, lung, colon, pancreatic cancer |
| Antitumorigenic; Oncogenic. | Down-expressed in breast, pancreas, and prostate cancer |
| miR-222 (1q22.2) | Overexpressed in CLL, thyroid papillary carcinoma, glioblastoma. Down-regulated in AML |
| miR-200 family (various) | Overexpressed in clear-cell carcinoma, metastatic breast cancer |
| miR-155 (1q22.1) | Down-regulated in prostate cancer |
| miR-17-5p (13q32) | Overexpression in testicular cancer |

Synthetic oligonucleotides are effective in vivo for most a couple of weeks, as has been demonstrated by experiments involving cancer cells engrafted in mice [221] and tail vein injection to mice [220]. To circumvent this limitation, miRNAs can be stably expressed through transcription of hairpin RNA from plasmid vector. Recently, artificial expression of a miRNA target sequence was shown to inhibit the miRNA function, presumably by titrating the miRNA away from endogenous targets [222,223]. Thus, it should be possible to apply such competitive inhibitors for long-term sequestration of a miRNA.

More recently, the Weinberg group has described another in vivo approach to modulating miRNA function with possible therapeutic implications. Their approach involved the use of a sponge vector [1], a vector expressing miRNA target sites design to saturate and endogenous miRNA and preventing it from regulating their natural targets [224]. In this case, miR-9 was identified as a pro-metastatic miRNA in breast cancer [1], and the miRNA sponge-mediated suppression of miR-9 in the highly metastatic 4T1 mouse mammary tumors cells reduced lung metastasis by 50%, although no effect was observed in the onset of the primary tumor.

Some chemical compounds alter the expression of a group of miRNAs [117]; therefore it may be possible to screen for drugs that could shift the miRNAome in a cancer cell toward that of a normal tissue. By modulating multiple miRNAs simultaneously, such a miRNAome modifying approach may be much more effective for
therapy than strategies that aim to regulate a single miRNA. Reconstitution of down-regulated miRNAs offers the theoretical edge of correcting the malignant defect by inducing small changes in miRNA gene dosage to a homeostatic level achieving substantive correcting the malignant defect by inducing small changes in miR-21 inhibition together with a secreted form of tumor necro-

sism factor-related apoptosis-inducing ligand, which results in a complete eradication of glioblastoma cells [230].

Collectively, preliminary results suggest that miRNAs could be useful for cancer therapy. However, there is still a significant gap between basic research on miRNAs and clinical application. Extensive preclinical and translational research is necessary to increase the efficacy and minimize the side effects of miRNAs-based therapy.

15. Conclusions

In summary miRNAs play critical roles in the tumorigenic process and altered miRNA expression is associated with the process of carcinogenesis and culminates in the development of cancer. Examples of miRNAs involved in human cancer are shown in Table 1.

MiRNAs profiles are significantly altered in numerous cancers affecting the cells transcriptome. Nonetheless, these small RNAs are also subjected to regulation by many cancer-associated pro-

teins such as p53 and c-Myc. Their expression patterns depend upon tumor origin, histotype, stage and grade. MiRNAs influence treatment responses and curability of tumors.

The complexity of the miRNA network and therefore, the possible alterations that they may suffer in the malignant process, is further intensified by the discovery of miRNA functions that fall outside their classic range. For example, there is evidence of miRNA-mediated increases in protein translation [23], nuclear import of miRNAs with distinctive hexanucleotide terminal motifs [76] and the secretion of miRNAs [231, 232]. Furthermore, an alternative processing pathways has been uncovered in Drosophila melanogaster and C. elegans that bypasses DROSHA and instead uses a splicing technique to generate miRNA precursors from short intronic sequences (mirtrons) [233, 234, 38]. Most importantly, we should be aware that miRNAs have also opened the door for the study of other non-coding RNAs in cancer, such as transcribed- untranslated regions (T-UCRs), that are also impaired in human tumors [235] and many times associated with promoter CpG island methylation silencing [236]. From a translational standpoint, profiles of pro-

moter hypermethylated miRNAs loci have started to show value as metastasis predictors [237]. In the future, miRNAs, and other non-coding RNAs, may serve as excellent biomarkers for early detection of tumors, and individual tailoring of therapeutic strategies.

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