MicroRNAs and cancer—new paradigms in molecular oncology
Massimo Negrini1, Milena S Nicoloso2 and George A Calin2

The ‘classic’ view of molecular oncology indicates that cancer is a genetic disease involving tumor suppressor and oncogenic proteins. However, in the recent years, it has been demonstrated that small regulatory non-coding RNAs (ncRNAs) named microRNAs (miRNAs) are involved in human tumorigenesis, thus revealing a new layer in the molecular architecture of human cancer. Gene expression studies revealed that hundreds of miRNAs are deregulated in cancer cells and functional studies clarified that miRNAs are involved in all the molecular and biological processes that drive tumorigenesis. Here, we summarize the recent advances in miRNA involvement in human cancer and illustrate the benefits of using these knowledge for medical practice. New diagnostic classifiers based on miRNAs will soon be available for medical practitioners and, even more importantly, miRNAs may become novel anti-cancer tools.

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MicroRNAs (miRNAs) constitute a large class of philogenetically conserved single stranded RNA molecules of 19–25 nt, involved in post-transcriptional gene silencing. They arise from intergenic or intragenic (both exonic and intronic) genomic regions that are transcribed as long primary transcripts. Primary transcripts undergo two processing steps that produce the short ‘mature’ molecule. The mature miRNA binds to specific regions of target mRNA transcripts and either destabilizes the target mRNA transcript or blocks its translation or both (for detailed reviews, see [1,2]).

Recent findings indicate that miRNAs are involved in the pathogenesis of all types of human cancers. Initially identified in B-cell chronic lymphocytic leukemia (CLL) [3*], miRNA alterations have since been detected in many types of human tumors (Table 1). The main mechanism of miRNome (defined as the full complement of miRNAs present in a genome) alterations in cancer cells is aberrant gene expression, which is characterized by abnormal expression levels of mature miRNAs.

In the present review we focus on the advances in understanding the roles of miRNAs in cancer reported in the past two years (for earlier studies, see these reviews [4–6]).

MicroRNAs as oncogenes and tumor suppressors
A growing amount of evidences proves that miRNAs can work as oncogenes (activating the malignant potential) or tumor suppressor genes (blocking the malignant potential) and they can affect all of the six hallmarks of malignant cells [7].

Self-sufficiency in growth signals
Tumor cells, in order to become independent from external growth factor signals and evade tissue homeostasis, constitutively activate different pathways sustaining cell proliferation and survival. In fact, RAS activating mutations are commonly observed in tumor cells. RAS oncogenic signaling is also granted by reduction of let-7, a well-documented post-transcriptional regulator of RAS [8*] and inversely correlated with RAS expression both in solid and hematological malignancies. Low expression of let-7 has been shown to be an indicator of poor prognosis in lung cancer patients (short post-operatory survival) [4] and in head and neck squamous cell carcinomas [9]. Interestingly, in acute myeloid leukemia the down-modulation of RAS by all-trans retinoic acid treatment (ATRA) relies on transcriptional induction of let-7 by NF-kB [10]. Let-7 down-modulation can also help tumor cells to grow in an anchorage independent manner and do not undergo apoptosis after they lose contact with the basal membrane. Low let-7 expression levels in tumors exert this effect [11,12] very probably via de-repression of the pleiothropic architectural transcription factor HMGA2, a major let-7 target [13], which contributes to multiple differentiation programs. The clinical relevance of let-7 anti-proliferative effects has been recently shown by let-7 induced tumor regression in vivo in murine lung cancer models [14,15] and these evidences are very promising for the development of let-7-based anti-cancer therapies.

miR-143 is found downregulated in several tumor histotypes, including B-cell malignancies [16] as well as breast,
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**Table 1**

<table>
<thead>
<tr>
<th>Rank</th>
<th>MicroRNA</th>
<th>Cluster</th>
<th>Chrom</th>
<th>Host gene</th>
<th>Associated cancer</th>
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<tbody>
<tr>
<td>1</td>
<td>miR-221</td>
<td>mir-221/mir-222</td>
<td>X</td>
<td>Intergenic</td>
<td>Stomach, colon, pancreas, liver, bladder, thyroid ca, glioblastoma</td>
</tr>
<tr>
<td>2</td>
<td>miR-21</td>
<td>–</td>
<td>17</td>
<td>TMEM49-005—exon 12</td>
<td>Breast, ovarian, tongue, liver, lung thyroid ca, glioblastoma</td>
</tr>
<tr>
<td>3</td>
<td>miR-191</td>
<td>mir-191/mir-425</td>
<td>3</td>
<td>C3orf60; intron 1</td>
<td>Breast, prostate, stomach, colon, pancreas, lung ca</td>
</tr>
<tr>
<td>4</td>
<td>miR-210</td>
<td>–</td>
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<td>Intergenic</td>
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<tr>
<td>5</td>
<td>miR-155</td>
<td>–</td>
<td>21</td>
<td>AP000223.5-001—exon 4</td>
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<tr>
<td>6</td>
<td>miR-222</td>
<td>mir-221/mir-222</td>
<td>X</td>
<td>Intergenic</td>
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</tr>
<tr>
<td>7</td>
<td>miR-34a</td>
<td>–</td>
<td>1</td>
<td>Intergenic</td>
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</tr>
<tr>
<td>8</td>
<td>miR-181b-1</td>
<td>mir-181a-1/mir-181b-1</td>
<td>1</td>
<td>RP11-31E23.1-001—intron 2</td>
<td>Breast, prostate, pancreas, liver, thyroid ca</td>
</tr>
<tr>
<td>9</td>
<td>miR-103-1</td>
<td>–</td>
<td>5</td>
<td>PANK3; intron 5</td>
<td>Breast, stomach, colon, pancreas, bladder ca</td>
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<tr>
<td>10</td>
<td>miR-181a-1</td>
<td>mir-181a-1/mir-181b-1</td>
<td>1</td>
<td>RP11-31E23.1-001—intron 2</td>
<td>Breast, tongue, liver, thyroid ca</td>
</tr>
<tr>
<td>1</td>
<td>miR-145</td>
<td>mir-143/mir-145</td>
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<td>Intergenic</td>
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<tr>
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<td>–</td>
<td>19</td>
<td>DNM2—intron 15</td>
<td>Ovary, prostate, colon, liver, lung ca</td>
</tr>
<tr>
<td>3</td>
<td>miR-143</td>
<td>mir-143/mir-145</td>
<td>5</td>
<td>Intergenic</td>
<td>Breast, ovary, prostate, colon, liver, lung ca</td>
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<tr>
<td>4</td>
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<td>5</td>
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<td>mir-125b-1/let-7a-2/mir-100</td>
<td>11</td>
<td>Intergenic</td>
<td>Breast, ovary, prostate, tongue, liver ca</td>
</tr>
<tr>
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<td>let-7a-1/let-7f-1/let-7d</td>
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<td>Breast, ovary, prostate, colon, liver ca</td>
</tr>
<tr>
<td>7</td>
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<td>1</td>
<td>Intergenic</td>
<td>Breast, ovary, prostate, colon, liver ca</td>
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<tr>
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<td>miR-138-2</td>
<td>–</td>
<td>16</td>
<td>Intergenic</td>
<td>Tongue, stomach, colon, pancreas, thyroid ca</td>
</tr>
<tr>
<td>9</td>
<td>miR-199a-2-5p</td>
<td>mir-214/mir-199a-2</td>
<td>1</td>
<td>DNM3—intron 14</td>
<td>Ovary, tongue, liver ca</td>
</tr>
<tr>
<td>10</td>
<td>miR-218-2</td>
<td>–</td>
<td>5</td>
<td>SLU7; intron 14</td>
<td>Prostate, stomach, colon, lung ca</td>
</tr>
</tbody>
</table>

*Rank was assessed on the basis of the number of published papers reporting the deregulation of the miRNA from 2002 to 2008.

Cervical [17], colorectal [18], bladder [19], and pituitary tumors [20]. Functionally, in cervical cancer miR-143 has been shown to suppress cell proliferation [21], and in colorectal cancer cells it does so by directly inhibiting KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncopogene homolog) and KRAS downstream signaling [22]. Furthermore, both in colorectal cancer [22] and in bladder cancer [19] miR-143 expression was found inversely correlated with KRAS expression levels.

miR-21, one of the most frequently upregulated miRNAs in solid tumors also participates in RAS oncogenic signaling. As recently shown, miR-21 is transcriptionally induced by AP1 downstream of RAS and exerts its oncogenic effect by keeping in check PTEN and PDCD4 [23]. Given that PDCD4 negatively controls AP1, AP1 induced miR-21 represents a positive feedback loop that sustains AP1 activity in response to RAS [23].

Cell cycle regulation is affected by miRNAs abnormalities in tumors as well. For example, miR-15b overexpression was shown to arrest cells in G0/G1 by targeting cyclin E1 [24]. As observed in glioma tumors, miR-15b is usually found reduced compared with normal brain tissue [24], therefore sustaining cell proliferation.

**Innsensitivity to antiproliferation signals**

Growth arrest is normally achieved by dephosphorylated proteins of the RB family, which sequester E2F transcription factors and inhibit the expression of genes required for cell cycle progression. E2F transcription factor activities are also controlled at the post-transcriptional level by a series of miRNAs. miR-17-5p, miR-20a, miR-106b, and miR-92 were found to inhibit E2F1 [25,26], whereas miR-20a was shown to inhibit E2F2 and E2F3 [27]. Furthermore, E2F activating transcription factors have been shown to regulate the expression of these clustered microRNAs [26,28]. Unbound E2Fs increase miR-17~92 and miR-106b~25 expression, and in turn these keep in check E2F levels and participate in the delicate equilibrium between cell proliferation and apoptosis, which is affected by alteration of E2F levels. Additionally, the miR-17~92 cluster preferentially shuts down the pro-apoptotic E2F1 in favor of the proliferative E2F3 transcriptional network. Upregulated miRNAs from these clusters are able to target apoptotic and growth inhibitory proteins such as BIM and p21 [26].

The miR-17~92 cluster is also essential for integrating signals during the G1 phase of the cell cycle and deciding whether a signal should be interpreted as proliferative or apoptotic [29]. In physiological conditions, the miR-17~92 cluster can limit MYC activation by dampening the E2F positive feedback loop. In tumors with MYC activation, miR-17~92 cluster protects cells from MYC-induced apoptotic E2F responses, leading to uncontrolled cellular proliferation.

The oncomiR-17~92 is found upregulated in many lymphoproliferative disorders [30] and solid cancer not only through MYC activation but also through...
Angiogenesis

Tumor cells turn on the ‘angiogenic switch’ to produce high amounts of pro-angiogenic factors and promote neovascularization. The most important angiogenic factor, VEGF, is highly expressed in most tumors, both solid and hematologic, and is induced by hypoxia. In tumor progression hypoxia has been found to contribute to the modulation of miRNA expression, partly by direct HIF-1 transcriptional activation of specific miRNAs [56]. These miRNAs have dual functions: on one hand, they participate in the angiogenic process, and on the other they aid the cell in engaging anti-apoptotic programs sustaining cell survival (e.g. miR-26, miR-107, and miR-210 inhibit caspase 3 activation). For example, miR-27a by repressing the proto-oncogene BCL6 [44,45], a p53 negative regulator, well known for being upregulated in lymphoproliferative disorders. In addition, miRNAs can act as regulators signaling downstream of TP53. In fact, miR-155 is responsible for silencing TP53 functions by directly repressing TP53INP1 [46], an important mediator of TP53 antioxidant and pro-apoptotic activities [47].

Other major pro-apoptotic miRNAs with reduced expression in tumors include miR-15a/16-1 cluster that repress the anti-apoptotic BCL2 protein and activate the intrinsic apoptotic program APAF-1-CASPASE-9-PARP [48], and miR-101, that among other important targets, such as the methyltransferase EZH2 [49], also silences the survival protein MCL1 [50].

Limitless replicative potential

Cellular senescence is a physiological withdrawal from the cell cycle in response to a multitude of different stress stimuli, including oncogene activation, and involving telomerase deregulation. MiRNA relevance to oncogene induced premature senescence has been addressed with a genetic miRNA-screening library: miR-373 and miR-372 were identified as capable of allowing transformation of primary cells harboring oncogenic RAS and wild-type p53, by neutralizing p53 mediated CDK inhibition through suppression of LATS2 [51]. Telomerase deregulation in tumors is also due to miRNA abnormalities. miR-138 and telomerase expression levels have been found inversely correlated in both anaplastic and papillary thyroid carcinoma [52] and the mechanistic explanation is through reduction of miR-138 expression levels in tumors, which can repress TERT mRNA translation.

When cells undergo senescence, p53 activated miRNAs are also important: it has been proven that the miR-34 family participates to the senescence program [53], through modulation, at least for miR-34a, of the E2F signaling pathway [54]. Furthermore, 15 miRNAs were found downregulated in senescent cells and in breast cancers harboring wild-type p53 and proved that these miRNAs are repressed by p53 in an E2F1-mediated manner [55].

Angiogenesis

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Evasion from apoptosis

Apoptosis is a physiological self-destruction cellular mechanism leading to removal of unwanted cells. The cancer associated genomic region 1p36, frequently lost or rearranged in many tumor types, including those originating from neural, epithelial, and hematopoietic tissues, contains as candidate tumor suppressors the pro-apoptotic miR-34a. The tumor suppressive function of this gene has been extensively studied in human neuroblastomas [35,36], where its loss synergizes with MYCN amplification. This is in line with the fact that miR-34a is a MYCN negative regulator [37]. miR-34a is also proficient to induce a cell cycle arrest and subsequent caspase-dependent apoptosis through BCL2 [36,37], E2F3 [35], and SIRT1 repression [38]. In addition, analysis of the transcriptome induced by miR-34 overexpression exhibits high similarity with that observed with p53 induction, being highly enriched for genes regulating cell cycle progression, apoptosis, DNA repair, and angiogenesis [39,40,**41**]. Indeed, miR-34 family members are direct transcriptional targets of p53 (for a review see [42]) and are essential for the correct execution of p53-dependent cellular responses. However, it should be considered that miR-34a pro-apoptotic effects seem to be cell type dependent; in fact, miR-34a was proven to increase in stress-induced renal carcinogenesis rat model, and its inhibition was proven to affect tumor cell proliferation [43].

miRNAs can also act upstream of p53. For example, miR-127, a miRNA epigenetically silenced in tumors, represses the proto-oncogene BCL6 [44,45], a p53 negative regulator, well known for being upregulated in lymphoproliferative disorders. In addition, miRNAs can act as regulators signaling downstream of TP53. In fact, miR-155 is responsible for silencing TP53 functions by directly repressing TP53INP1 [46], an important mediator of TP53 antioxidant and pro-apoptotic activities [47].

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has been shown that VEGF is also restrained at the post-transcriptional level by miRNAs. On one hand, miR-126 was found to directly repress in *in vitro* and *in vivo* lung cancer cell models VEGF-A expression and to induce a G1 cell cycle arrest with an overall reduction of tumor volume [59]. On the other hand, miR-126 expression was found to be enriched in endothelial cells during angiogenesis and to repress negative regulators of the VEGF pathway (SPRED1 and PIK3R2) [60,61], therefore exerting opposite roles according to cell context. Additionally, miRNAs downregulated in hypoxic conditions, such as miR-16, miR-15b, miR-20a, and miR-20b are able to directly modulate VEGF expression levels as well. This creates a positive feed-forward loop in which hypoxic repressed miRNAs reinforce the expression levels of a potent pro-angiogenic hypoxic induced growth factor, as VEGF [62].

**Invasion and metastasis**

The metastatic process starts with the acquisition of an invasive behavior that allows cells to detach from the primary tumor, enter the blood or lymphatic vasculature and spread to distant organs. It was revealed that upregulation of miR-10b promotes invasion and metastasis. Twist, a metastasis-promoting transcription factor, could induce miR-10b expression, whereas HOXD10, a homeobox transcription factor that promotes or maintains a differentiated phenotype in epithelial cells, was shown to be a target of miR-10b and to be expressed at low level in metastatic tumors. Consequently, the levels of RhoC, a G-protein involved in metastasis that is repressed by HOXD10, increase significantly in response to miR-10b expression [63**].

Distinct lines of evidence revealed that miR-373 and miR-520c are also metastasis-promoting miRNAs. Their pro-invasive and pro-migratory effect was initially studied in *in vitro* and *in vivo* breast cancer models and explained via direct suppression of CD44. CD44 encodes a cell surface receptor for hyaluronan, [64**] and is consistently reduced in metastatic breast, colon, and prostate cancer. The pro-invasive function of miR-373 and miR-520c via CD44 regulation has been further confirmed in a prostate cancer model [65].

MiRNAs can also be metastasis suppressors, as it was first revealed for miR-335, miR-126, and miR-206. Clinically, low expression of miR-335 or miR-126 in breast cancer patients was significantly associated with poor metastasis-free survival. Experimentally, the knockdown of SOX4 and tenascin C (TNC) diminished breast cancer cells *in vitro* invasive ability and *in vivo* metastatic potential, indicating these genes as crucial effectors of the metastatic program downstream of miR-335 [66**].

miR-21, besides controlling cell survival and proliferation, is also a master regulator of the metastatic process by directly modeling the cell cytoskeleton via TPM1 suppression [67,68], and by indirectly regulating the expression of the pro-metastatic UPAR (via maspin and PDCD4 direct suppression) [68] and of matrix metalloproteinases (via RECK, TIMP3 [69], and via PTEN [70] direct suppression).

Interestingly, the pleiothropic putative tumor suppressor miR-34a, in addition to repressing genes involved in G1 arrest, apoptosis, and senescence, has been shown to participate in the regulation of tumor cell scattering, migration, and invasion via downregulation of c-MET and its downstream signaling cascades [71].

All the members of the miR-200 family together with miR-205 were found significantly down-modulated during the epithelial–mesenchymal transition (EMT) (for a review see [72]), a developmental process through which cells loosen their cell–cell and cell–matrix contacts and switch from a collective invasion pattern to a detached and disseminated cell migration method. These miRNAs are able to regulate the expression of key mediators (i.e. TGFB1 and ZEB1/2) involved in the EMT and tumor metastasis. Recent observations demonstrated that not only miR-200 family regulates ZEB1/2 expression but also ZEB1 regulates miR-200 family transcription [73*,74*], thus establishing a complex regulatory loop that may ensure the tight control of the EMT process.

**Sequence variations in microRNAs and microRNA targets as cancer predisposing factors**

There are at least two lines of evidence indicating that miRNA genetic changes may increase cancer susceptibility.

**Germline mutations in microRNAs as cancer predisposing or risk factors**

After the identification of germline mutations in familial CLL cases [75], several studies investigated sequence variations in miRNAs and/or interactor sites within target miRNAs (Table 2). Germline and somatic mutations in miRNAs were identified in about 10% of patients with CLL. A germline mutation in the primary transcript of the tumor suppressor miR-16-1, which is located 7 nt downstream from the pre-miRNA, could lead to reduced expression of miR-16 and miR-15a. The same effect was observed for a mutation located 6 nt downstream from the murine miR-16 gene in the New Zealand Black (NZB) strain of mice, susceptible to the development of CLL [76]. Of note, one of two patients with CLL harboring the mutation was a member of a family with a history of cancer. Taken together, these two studies, one of human CLL and the other of a mouse model of resembling human CLL, indicate that miR-16 is the first miRNA proven to be involved in cancer predisposition.
More recently, it has been reported in a prostate cancer patient that a germline G-to-A mutation 19 nt downstream from the miRNA let-7e, a member of the largest family of tumor suppressor miRNAs, leads to a significant reduction in the gene expression in vivo [77], probably contributing to the tumorigenic process.

### Single nucleotide polymorphisms in miRNAs or target sites for miRNAs are influencing the cancer risk

Researchers found new SNPs and novel mutations distributed in the regions of primary miRNAs, precursor miRNAs, and even mature miRNAs that are correlated to lung cancer survival [78], common genetic variants in pre-miRNAs that are associated with increased risk of breast cancer in Chinese women [79], and a common SNP in pre-miR-146a that decreases mature miRNA expression and predisposes individuals to papillary thyroid carcinoma [80] (Table 2). In addition to modifying miRNA mature levels, owing to interference with the primary transcript maturation process, SNPs inside miRNA precursors can affect the sequence and therefore the function of miRNAs produced by the passenger strand (‘star’ miRNAs), as shown for the two minor miR-146a* allelic variants, which presented a different set of repressed genes. In fact, it was found that expression of miR-146a SNP in heterozygosity was linked to extensive DNA-damage response following abnormal regulation of apoptotic pathways, potentially explaining the higher predisposition to papillary thyroid carcinoma of heterozygous patients [81].

Recently, let-7 complementary sites (LCS) were sequenced in the KRAS 3’ untranslated region and the LCS6 variant allele was found significantly associated with an increased risk for non small cell lung carcinoma among moderate smokers, representing a new paradigm for let-7 miRNAs in lung cancer susceptibility. Functionally, the variant allele results in KRAS overexpression in vitro [82**].

Another SNP inside a putative miRNA target (rs2747648 in ESR1) was computationally predicted to reduce the expression of ESR-1; consistently, this SNP was found to be clinically associated with a stronger risk of familial breast cancer in pre-menopausal women [83], further supporting the hypothesis that SNPs inside miRNA target sites can participate to tumor predisposition.

### Circulating microRNAs as novel diagnostic markers

As miRNAs are active players in human oncogenesis, an ever-growing number of reports are proving that miRNAs may represent novel diagnostic and prognostic tools for cancer stratification. For example, it was recently reported that high miR-21 expression is associated with poor survival and poor therapeutic outcome in colon adenocarcinoma [84], and a growing number of studies identified miRNA expression in human tumors as diagnostic or prognostic markers (for a review see [85]).

More recently, miRNA usefulness as diagnostic markers was further expanded by studies performed in human plasma or serum. With the exception of leukemias, where malignant cells are easily available, tissues for profiling of solid cancers are obtained either by biopsy (and amount and availability is every time a concern) or by surgery (and usually this is on late stage cancers, when the diagnostic and prognostic predictions are not anymore of interest, at least for the patient!). Therefore, studies that demonstrate the diagnostic and prognostic usefulness of circulating microRNAs are of high interest. Recently, the existence of specific expression profiles of serum miRNAs for lung cancer, colorectal cancer, and diabetes compared to healthy subjects was proved [86]. In another study, miR-17-5p and miR-92 were found significantly upregulated in plasma samples from colorectal cancer patients and underwent a significant reduction after surgery [87]. In addition, miR-92 was able to distinguish colorectal cancer from gastric cancer, inflammatory bowel disease, and normal subjects [87]. In renal cell carcinoma patients instead, the levels of circulating miRNAs were predictive of...
malignancy and survival [88]. Interestingly, other studies reported that serum levels of miR-21 were associated with relapse-free survival and had the potential as a diagnostic biomarker for diffuse large B cell lymphoma [89]. Likewise, serum levels of miR-141 were capable to distinguish patients with prostate cancer from healthy subjects [90]. Given the fact that most of all current approaches to cancer screening are invasive and unable to detect early-stage disease, it would be important to determine when tumor-related circulating miRNAs can be detected in bloodstream during disease evolution.

**MiRNA-based cancer gene therapy**

RNA inhibition can be used to treat cancer patients in two ways: (a) by using RNA or DNA molecules as therapeutic drugs against messenger RNA of genes involved in the pathogenesis of cancers and (b) by directly targeting ncRNAs that participate in cancer pathogenesis. The specific tools used to accomplish these goals are presented in Table 3.

The use of miRNAs as potential drugs is still at preclinical stage, and no clinical or toxicological studies are published yet. miRNA mimics were shown to induce cell death and significant reduction of tumorigenic potential for miR-15a/16 cluster in a leukemia cell model [48] and for the members of miR-29 family in a lung cancer model [91].

The ‘antagomir’ represents a RNA therapeutic molecule originally designed to inhibit miRNAs [92]. The proof-of-principle for the potential anti-cancer activity of antagomirs was shown in a neuroblastoma model [93], where overexpression of miR-17/C24 cluster augments in vivo tumorigenesis of MYCN-not-amplified neuroblastoma cells. Tumors subcutaneously induced in nude mice were treated with antagomir-17-5p for two weeks, which resulted in inhibition of tumor growth and in complete regression in 30% of cases. The control antagomir did not affect tumor development.

Recently, studies performed in mouse and African green monkeys models assessed safety and efficacy of locked nucleic acid (LNA)-mediated microRNA silencing [94]. Efficient silencing of miR-122 was achieved by three doses of 10 mg/kg LNA-antimiR, leading to a long-lasting and reversible decrease in total plasma cholesterol without any evidence for associated toxicities or histopathological changes in the liver of the animals. Thus, by proving feasibility, safety and efficacy for the use of anti-miRNA oligonucleotides (AMOs) in a preclinical setting, these studies established the basis for their use as therapeutic molecules in clinical trials.

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**Table 3**

<table>
<thead>
<tr>
<th>Description</th>
<th>Definition</th>
<th>Mechanism of action</th>
<th>Therapeutic stage in cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antisense oligonucleotides (ASOs)</td>
<td>A single stranded, chemically modified DNA-like molecule that is 17–22 nt in length and complementary to a selected messenger RNA.</td>
<td>Watson–Crick binding, leading to RNase-H mediated cleavage of the mRNA of target gene and thereby specifically inhibit expression of that gene.</td>
<td>Clinical trials phase II and III</td>
</tr>
<tr>
<td>Ribozymes or DNAzymes</td>
<td>A RNA enzyme is an RNA molecule that can catalyze a chemical reaction. A deoxyribozyme is a catalytic DNA that specifically cleaves the target RNA.</td>
<td>Watson–Crick base pairing to a complementary target sequence, followed by site-specific cleavage of the substrate and finally release of the cleavage products.</td>
<td>Clinical trials phase I and II</td>
</tr>
<tr>
<td>siRNAs</td>
<td>A double strand (ds) RNA homologous to an mRNA of a target gene.</td>
<td>Watson–Crick base pairing, leading to RNAse-H mediated cleavage of the mRNA of the target RNA.</td>
<td>Preclinical studies</td>
</tr>
<tr>
<td>MicroRNAs mimics</td>
<td>A small single-strand 19–24 nt RNA produced from the cleavage of a hairpin structure by RNAsell enzymes</td>
<td>Watson–Crick binding, leading to RNAse-H mediated cleavage of the mRNA of the target RNA.</td>
<td>Clinical trials phase I</td>
</tr>
<tr>
<td>LNAs anti-miRs and antagomirs</td>
<td>The LNAs anti miRNAs represents LNA modified ASOs specifically designed to block the function of microRNA.</td>
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Perspectives
There are no doubts that miRNAs are important cancer players. Main advances in this competitive field in next future will come from at least three areas: first, the identification of functional feedback and feed-forward loops involving transcription factors – microRNAs – gene targets, which will provide a wider picture of how cells become malignantly transformed, multiply, and invade patient’s body; second, the identification of the clinical conditions in which miRNAs may become useful markers for early diagnosis, improved prognosis and as therapy indicators; third, the definition of the best conditions for the use of miRNAs and/or their inhibitors as anti-cancer agents. A great challenge will be to identify the right set of patients that could benefit from such advanced types of therapies.

Acknowledgements
Negrini is supported by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC), Ministero della Università e Ricerca, Ministero della Sanità, and by project NOBEL from Fondazione Cariplo, Milan, Italy; Calin is supported as a Fellow of The University of Texas M. D. Anderson Research Trust, and as a Fellow of University of Texas System Regents Research Scholar and as a Ladvejgaard Regents Research Scholar Fund. This study was funded also by an Institutional Research Grant (IRG) and by a CCSG (New Faculty Award) to GAC. We apologize to our colleagues whose work was not cited owing to space limitations.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This article describes a negative feedback loop between miR-106b-25 cluster on chromosome 7q22.1 and E2F1. On one hand E2F1 transcriptionally induces miR-106b-25 cluster, on the other miR-106b and miR-93 cluster post-transcriptionally repress E2F1 expression levels, ensuring a tight control of cell cycle progression.
MicroRNAs and cancer—new paradigms in molecular oncology

Negrini, Nicoloso and Calin


This article together with reference [28] shed light on the complex regulatory feedback loops governing miR-17-92 cluster correct expression.


This article together with reference [27] shed light on the complex regulatory feedback loops governing miR-17-92 cluster correct expression.


This study and reference number [40] identified miR-34 as a major miRNA regulated by TP53.


74. This work and reference [74] identify a complex auto-regulatory loop between ZEB1 transcription factor that is an important mediator of EMT and miR-200 family that instead sustain an epithelial cell phenotype. ZEB1 represses miR-200 family members, which in turn when overexpressed repress ZEB1, ZEB2, and also TFGFB2.


83. The authors identified miR-21 as a marker for the response to chemotherapy.


89. The authors identified miR-21 as a marker for the response to chemotherapy.


